



## 2015 ASPHO and PBMTC Abstracts

### Wednesday, May 6 at 6:30-7:30pm

Plenary Platform Session: 4001-4002.....2

### Thursday, May 7 at 4:15pm-5:55pm - Concurrent Platform Session I

(4003-4006) Leukemia I .....3

(4007-4010) Hematology II .....6

### Friday, May 8 at 11:30-12:30pm - Concurrent Platform Session II

(4013-4016) Solid Tumors.....11

(4017-4021) Quality Improvement and other Interesting Topics .....15

### Saturday, May 9 at 9-10:00am - Concurrent Platform Session III

(4022-4025) Leukemia II .....19

(4026-4029) Hematology II .....22

### Posters

#### Thursday, May 7 at 6-7pm (odd numbered)

501-527 Bone Marrow Transplant .....26

529-555 Hematology (including: Anemia, Diamond Blackfan Anemia, Fanconi, Neutropenia, Schwachman, Stem Cells, and Transfusion).....50

557-581 Hemostasis (including: Hemophilia, Idiopathic Thrombocytopenic Purpura, Platelets, Thrombosis, and Vascular Anomalies).....73

583-595 Survivorship.....95

597-687 Case Reports (including: Hematology, Leukemia, and Lymphoma) .....106

689-729 Leukemia (including: Clinical, Histiocytosis, Infection, Lymphoma, T-ALL) .....181

731-763 Solid Tumors (including: Sarcoma, Clinical).....216

#### Friday, May 8 at 11:30-12:30pm (even numbered)

500-532 Quality Improvement (including: Infection, Psychology, Training, Training, and Transfusion) .....25

534-598 Red Cells (including: Sickle Cell, Thalassemia) .....54

600-690 Case Reports (including: Vascular Anomalies, Histiocytosis, Solid Tumors, and Brain) .....108

692-732 Leukemia (including: ALL, AML, CAR, and Clinical) .....183

734-764 Solid Tumors (including: Kidney, Neuroblastoma, Thyroid, and Brain).....218

PBMTC Posters ..... 5000-5041

## USE OF LEVOFLOXACIN PROPHYLAXIS TO REDUCE INFECTIONS IN PATIENTS WITH AML

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**Background:** Bacterial sepsis continues to be a leading cause of morbidity and toxic death in children receiving chemotherapy for acute myelogenous leukemia (AML). Death due to bacterial infection has been reported in 4-7% of AML patients in various trials. Adult studies have found decrease in mortality in patients receiving quinolones prophylaxis in times of profound neutropenia. In small studies of pediatric patients, antibiotic prophylaxis has also been associated with decreased fever admissions and bacteremia. In our efforts to decrease blood stream infections at Children's Healthcare of Atlanta (Atlanta, GA, United States), we standardized the use of levofloxacin prophylaxis in our AML patients beginning in June 2012.

**Objectives:** To determine the effectiveness of levofloxacin in reducing bacterial infections during times of chemotherapy-induced neutropenia in patients with AML.

**Design/Method:** This study is a retrospective chart review that involves the analysis of primary AML patients during initial therapy at Children's Healthcare of Atlanta between 9/29/2010 to 9/1/2014. Patients with Down syndrome associated AML were excluded.

**Results:** Thirty nine patients received initial treatment for AML during this time period. These patients represent 132 courses of cytarabine-containing therapy. Levofloxacin prophylaxis was given in 80 of these cycles. The incidence of infections found in the population is shown in Table 1.

**Conclusion:** Levofloxacin prophylaxis in AML patients decreased the number of both strep viridans and gram negative infections during periods of neutropenia. We did not see an increase in fungal infections during this period.

Infection type by course	Levofloxacin (N=80)	No Levofloxacin (N=52)	p-value
Strep viridans	10 (12.5%)	15 (28.85%)	0.024
Gram positive infections	23 (28.75%)	20 (38.46%)	0.213
Gram negative infections	0	5 (9.62%)	0.024
Fungal infection	2 (2.5%)	5 (9.62%)	0.112
Any infection Any site	27 (33.75%)	26 (50%)	0.067

## INHIBITION OF LEUKEMIA-CELL CALCINEURIN PROMOTES T CELL-MEDIATED SUPPRESSION OF LEUKEMOGENESIS

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**Background:** Immunologic evasion is one of the hallmarks of cancer, but the mechanisms by which leukemia evades the immune system are incompletely understood. Previous experiments have demonstrated a role for calcineurin (Cn) in the development of leukemia. We hypothesized that leukemia-cell Cn mediates an immune evasion program during leukemogenesis.

**Objectives:** To investigate the role of Cn in the pathogenesis of acute leukemia.

**Design/Method:** Ppp3r1, an essential subunit for Cn function, was knocked down with shRNA (shCn) in luciferase-expressing BCR-ABL1+ ALL cells. shCn cells or controls expressing a non-silencing shRNA (shNS) were injected into un-irradiated, syngeneic immune-competent mice, Rag-1 deficient mice or TCR $\alpha$  knockout mice. Disease burden was measured by luciferase activity. Bone marrow (BM) cell subsets were analyzed by flow cytometry, and ALL cells were sorted from mice for gene expression analysis by RNA-seq. We also performed flow cytometry of ALL cells for cell surface expression of molecules with known immunomodulatory function.

**Results:** Engraftment of shNS and shCn ALL was detected between days 3 and 7 in nearly all mice, and disease burden increased exponentially in recipients of shNS ALL. However, by day 10, the disease burden in all recipients of shCn ALL was undetectable. shCn recipients had significantly longer overall survival (Median 17d v. 77d; P=0.002), and knockdown of Ppp3r1 was lost at relapse. Transplantation of shCn cells into Rag1 and TCR $\alpha$  mutant recipients did not result in leukemia regression or prolonged survival. Analysis of BM at day 7 revealed that recipients of shCn ALL have a significant increase in the percentage of CD3+ cells (P=0.01) and the ratio of CD8/CD4 T cells (P<0.001) in the BM. RNA-Seq revealed differential expression of 236 genes (FDR P<0.05), and pathways analyses suggested that cytokine-cytokine receptor genes are enriched in this set (P=0.002). Cell surface staining for MHC1, PD-L1, PD-L2, CD80, and CD86 did not reveal differences in expression between shNS and shCn ALL cells.

Preliminary experiments suggest that NFAT is not the downstream target of Cn in this context.

**Conclusion:** Leukemia-cell Cn mediates a potent immune evasion mechanism during leukemogenesis. Ongoing experiments are designed to determine downstream molecules responsible for Cn dependent immune evasion.

Platform Session #4003

## CHIMERIC ANTIGEN RECEPTOR (CAR)-MODIFIED T CELLS INDUCE DURABLE REMISSIONS IN CHILDREN WITH RELAPSED/REFRACTORY ALL

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**Background:** Relapsed/refractory pediatric acute lymphoblastic leukemia (ALL) poses a substantial therapeutic challenge. Targeted immunotherapy using chimeric antigen receptor (CAR)-modified T cells combines the specificity of an antibody's single chain variable fragment (scFv) with intracellular T cell signaling domains, delivering T cells with potent cytotoxicity to antigen-expressing tumor cells. We previously reported complete remissions and prolonged persistence in children and adults with ALL treated with CD19-specific CART cells (CTL019). We now report on outcomes and longer follow-up of the first 40 children with relapsed/refractory ALL treated with CTL019.

**Objectives:** Establish the safety and efficacy of CTL019 for children with relapsed/refractory CD19+ ALL.

**Design/Method:** T cells collected from the patient were transduced with a lentiviral vector encoding a CAR composed of anti-CD19 scFv, CD3z, and 4-1BB domains, activated/expanded ex vivo with anti-CD3/anti-CD28 beads, cryopreserved, and then infused. 35/40 patients received lymphodepleting chemotherapy the week prior to cell infusion.

**Results:** Of 40 patients aged 5-22y (median 11y) with CD19+ ALL, 33 had detectable disease prior to CTL019 cell infusion, while 7 were MRD(-). 28 had relapsed after prior SCT. A median of  $3.6 \times 10^6$  CTL019 cells/kg ( $0.98-17 \times 10^6$ /kg) were infused in 1-3 doses. At assessment 1 month after infusion, 37/40 (93%) were in a complete remission (CR). MRD <0.01% by flow cytometry was achieved in 34 patients. With median follow-up 7 mo (1-31 mo), 26 patients had ongoing CR, with only 5 receiving subsequent therapy (1 DLI, 4 SCT), 6-month EFS was 72% (95% CI, 59-89%), and OS was 77% (95% CI, 64-92%). CTL019 cells were detected in the CSF and 4 patients with CNS2a disease experienced ongoing CRs in CSF. Ten patients subsequently relapsed, 5 with CD19(-) disease. CTL019 persistence was accompanied by B cell aplasia, which continued up to 30 months in patients with ongoing CR. Cytokine release syndrome (CRS) was seen in almost all (37) patients. Severe CRS requiring hemodynamic or respiratory support occurred in 33% of patients, was associated with high disease burden and elevations in CRP, ferritin, and IL-6, and was rapidly reversed with the anti-IL6R agent tocilizumab.

**Conclusion:** Single-agent CTL019 immunotherapy can induce potent and durable responses in patients with relapsed/refractory ALL.

Platform Session #4004

## AML IMMUNOTHERAPY WITH CD123-ENGAGER T CELLS

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**Background:** Novel treatments are needed for patients with AML and immunotherapies have the potential to fulfill this need. Adoptive transfer of tumor-specific T cells is one promising approach; however, infused T cells do not redirect resident T cells to tumors. To overcome this, we have genetically modified T cells to produce a secretable, bispecific T-cell engager (ENG-T cells). Consistent synthesis of engagers by T cells should be superior to the direct infusion of the recombinant bispecific antibody, because these recombinant proteins have short half-lives and they do not accumulate at tumor sites.

**Objectives:** The goal of this project was to generate and characterize T cells secreting IL3Ra (CD123) and CD3 bispecific T-cell engagers (CD123-ENG T cells).

**Design/Method:** CD123-ENG T cells were generated by transducing T cells with a retroviral

vector encoding a CD123-specific T cell engager consisting of an scFv recognizing CD123 linked to an scFv recognizing CD3. Effector function of CD123-ENG T cells was evaluated *in vitro* and in a xenograft model.

**Results:** Mean transduction efficiency was 76% (range 49-95%), and coculture of CD123+ cells (MV-4-11, MOLM-1, KG1a, K562-CD123) with CD123-ENG T cells resulted in T-cell activation as judged by cytokine secretion. In contrast, CD123-negative cells (K562) did not activate CD123-ENG T cells. Likewise, control ENG-T cells were not activated by CD123+ cells. Antigen-dependent recognition was confirmed with cytotoxicity assays ( $p < 0.05$ ). Since CD123 is expressed on normal hematopoietic progenitor cells (HPCs), we evaluated the ability of CD123-ENG T cells to recognize normal HPCs in colony formation assays. Only at high CD123-ENG to HPC ratios did we observe a decline in colony numbers, indicating that CD123+ AML cells can be targeted while preserving normal HPCs. *In vivo*, CD123-ENG T cells had potent anti-tumor activity in the KG1a/NSG xenograft model resulting in a significant survival advantage of treated animals in comparison to untreated mice and mice that received Control-ENG T cells ( $p = 0.002$ ).

**Conclusion:** We have generated CD123-ENG T cells that can direct bystander T cells to CD123+ AML in an antigen-specific manner. These CD123-ENG T cells have powerful anti-AML activity *in vivo*, presenting a promising addition to currently available AML therapies.

Platform Session #4005

## ROLE OF KLF4 IN T CELL LEUKEMIA

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**Background:** T-cell Acute Lymphoblastic leukemia (T-ALL), an aggressive hematologic malignancy, has an event free survival of 70% despite introduction of intensive, multi-agent chemotherapy. Our capacity to increase the overall survival rate is dependent on an improved understanding of disease mechanisms and on the characterization of novel therapeutic targets. Among a myriad of tumor suppressors involved in carcinogenesis, KLF4 has emerged as a bifunctional transcription factor. In normal T cells, KLF4 has a role in development and differentiation, controlling cell cycle progression by regulating expression of genes encoding components of the cyclin/cyclin-dependent kinase (cdk) complex, including p27<sup>Kip1</sup>. However, the function of KLF4 and its target genes in T cell leukemogenesis remains unexplored.

**Objectives:** To demonstrate an obligate role for KLF4 in the suppression of T cell leukemogenesis and the requirement for repression of p27<sup>Kip1</sup> in this process.

**Design/Method:** The expression of KLF4 and of p27<sup>Kip1</sup> was quantified in patient samples and T-ALL cell lines (Jurkat, Molt4, CEMC1, CEMC7) using standard real time Quantitative PCR, Western blot and Flow cytometric analysis and compared to normal human thymic tissue. A murine model with T lineage restricted conditional deletion of KLF4 was established using cre-recombinase gene targeting strategy in which Lck promoter drives expression of the cre-recombinase gene and a FACS analysis and a CFSE proliferation analysis was performed.

**Results:** Reduced expression of KLF4 and p27<sup>Kip1</sup> mRNA and protein was observed in T-ALL cell lines and patient samples. Flow cytometry demonstrated a significant decrease in KLF4 expression level in the patient samples. The murine model creates a distinct phenotype that includes a decrease in the number of single positive CD4+ thymocytes and peripheral CD4+ T

cells. CD4+ and CD8+ cells in the spleen of these mice have reduced proliferative capacity upon TCR stimulation.

**Conclusion:** KLF4 has an essential role in T cell development and differentiation. A concomitant loss of KLF4 and p27<sup>Kip1</sup> expression is associated with leukemogenesis in human T cell ALL.

Platform Session #4006

## **RESISTANCE TO CYTARABINE IN ACUTE MYELOID LEUKEMIA BY EXOSOMES DERIVED FROM PRIMARY BONE MARROW STROMAL CELLS.**

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**Background:** Stromal cells in the bone marrow are reprogrammed by leukemia, which influences drug resistance through unclear mechanisms. Extracellular vesicles, such as exosomes, contribute to paracrine signaling in several malignancies, but have not yet been fully described as a means of chemoresistance in AML. Here, we illustrate genetic reprogramming of stromal cells from AML patients, and show that these cells release exosomes enriched for a subset of cytokines with a role in chemo-resistance. We go on to show that stromal cells from AML and from healthy patients release exosomes that protect leukemia cells from cytarabine, a cornerstone of AML therapy, despite the observed differences in cytokine content.

**Objectives:** To evaluate the effect of exosomes released from reprogrammed AML or healthy bone marrow stromal cells on resistance to cytotoxic chemotherapy by high-risk Acute Myeloid Leukemia (AML) cells.

**Design/Method:** Marrow stromal cells were isolated from 20 AML (AML-BMSCs) and 4 healthy patients (N-BMSCs). We assessed cellular mRNA levels using qRT-PCR, and we used ELISA and a multiplex bead-based assay to quantify cytokine levels in exosomes. As a model for high-risk AML, MOLM-14 (FLT3-ITD+) AML cells were co-cultured with exosomes from AML-BMSC's or N-BMSCs, then exposed to increasing concentrations of the nucleoside analog cytarabine. Viability was measured using the tetrazolium-based MTS assay.

**Results:** AML-BMSCs show altered gene expression (SCF, CXCL-1, IGFBP4, Gas-6, CXCL-10, ANGPTL4) and an enriched subset of cytokines in their exosomes (GCSF, TGF- $\beta$ 1, TNF- $\alpha$ ), several of which have been implicated in chemo-resistance, relative to N-BMSCs. In correlative functional studies, 100% of exosomes from *both* stromal populations (total n=10; 6 from AML-BMSC and 4 from N-BMSC) conferred significant protection of AML cell viability in the context of cytarabine treatment. Consistent with our previous reports, only AML-BMSC exosomes showed protection against the FLT3 inhibitor, AC220.

**Conclusion:** Our data suggest that stromal exosome trafficking is a candidate mechanism for resistance to cytotoxic chemotherapy in the AML niche, and that this protection is independent of the reprogramming of AML-BMSCs. Further investigation into the differential effects observed against cytotoxic and targeted therapy by stromal exosomes may uncover novel pathways of resistance with therapeutic potential.

Platform Session #4007

## **A NOVEL MECHANISM OF FETAL HEMOGLOBIN INDUCTION VIA IKAROS**

## SUPPRESSION IN SICKLE CELL ANEMIA BY THE IMiD COMPOUND POMALIDOMIDE

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**Background:** Reactivation of fetal hemoglobin (HbF) is the therapeutic Holy Grail for the treatment of  $\beta$ -hemoglobinopathies. The second-generation immunomodulatory drugs (IMiD), pomalidomide and lenalidomide, have been identified as inducers of HbF, but their mechanism of action is unknown.

**Objective:** We sought to characterize their effect on erythropoiesis as well as identify IMiD targets using an *in vitro* 3-phase culture system.

**Design/Method:** CD34+ cells isolated from sickle cell disease (SCD) patients and normal controls were expanded for 4 days, and differentiated to red cells in the presence of 1  $\mu$ M pomalidomide or 10  $\mu$ M hydroxyurea, the sole FDA approved drug for SCD, over 16 days. HbF production was analyzed by flow cytometry, and high performance liquid chromatography;  $\gamma$ -globin chain production and transcription factor levels by qPCR and western blot. Erythropoiesis was monitored by flow cytometric analysis of glycophorin A, band 3, and  $\alpha$ 4-integrin expression as well as morphologically by Giemsa stain at days 7, 11, 14 and 16. When needed, 5  $\mu$ M MG132 was used to inhibit the proteasome.

**Results:** Pomalidomide treated cultures demonstrated a transient delay in differentiation, whereas hydroxyurea accelerated erythropoiesis. Both treatments did not significantly affect terminal differentiation, as cultures at day 16 possessed similar numbers of enucleated reticulocytes.

Pomalidomide and lenalidomide were recently shown to degrade multiple myeloma cell *Ikaros* via the ubiquitin-proteasome pathway. As *Ikaros* regulates the fetal-to-adult globin switch in development, we posited that this mechanism might be preserved in red cell maturation. Indeed, *Ikaros* expression is ablated as early as 1 hour of pomalidomide treatment, and is sustained throughout the duration of the culture. Moreover, *Ikaros* expression is rescued by treatment with MG132, a proteasome inhibitor. To expand these findings, we investigated the expression of other known  $\gamma$ -globin repressors including BCL11A, Mi2 $\beta$ , KLF1, SOX6, and lysine specific demethylase-1 (LSD1). Pomalidomide was found to decrease the expression of these factors at day 4; however, only BCL11A and SOX6 expression levels remained diminished beyond this time point.

**Conclusion:** Our results suggest that pomalidomide induces HbF through *Ikaros* degradation, causing alterations to downstream transcription factor networks.

Platform Session #4008

## EFFICACY OF RED CELL EXCHANGE, PARTIAL MANUAL EXCHANGE, AND SIMPLE TRANSFUSION, CONCURRENTLY WITH IRON CHELATION THERAPY, IN REDUCING IRON OVERLOAD IN CHRONICALLY TRANSFUSED SICKLE CELL PATIENTS

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**Background:** Chronic transfusion therapy (CTT) is indicated for primary and secondary stroke prevention in children with Sickle Cell Disease (SCD). Three common CTT modalities employed include: simple transfusion (ST), partial manual exchange (PME), and automated red cell exchange (RCE). Iron overload and RBC alloimmunization complicate CTT. Although small case series have demonstrated that chronic RCE in combination with iron chelation therapy is effective in stabilizing or decreasing ferritin levels, comparing the effect of ST, PME, and RCE on Liver Iron Concentration (LIC) over time has not been well described in SCD patients on iron chelation.

**Objectives:** To compare the efficacy of minimizing iron overload (via change in Ferritin and LIC over time), alloimmunization rates, and maintenance of 30% HbS in three transfusion modalities in SCD patients on CTT and deferasirox.

**Design/Method:** Medical records of SCD patients on CTT and deferasirox (25-35 mg/kg/day) were retrospectively reviewed over a 44-month period. At least 6 months of hematologic data were required for patient inclusion per CTT modality. Average HbS%, change in ferritin and LIC, and alloimmunization rate among CEK-matched transfusions was determined for each patient. Changes in ferritin and LIC were determined by calculating the slope of ferritin and LIC rise/decline (ng/mL/month and mg/gm dry weight/year respectively).

**Results:** Thirty-six patients were included (20 ST, 6 PME, 10 RCE). The average HbS% for ST, PME, and RCE was 33%, 36%, and 34% respectively. Average changes in ferritin were +2.4 ng/mL/month (range -306 to +101), +19.0 ng/mL/month (range -42 to +106), and -61 ng/mL/month (range -161 to +17) in patients receiving ST, PME, and RCE respectively ( $p < .0001$  using random effects model). In 23 patients with  $\geq 2$  LIC measurements, changes in LIC significantly differed by treatment: +1.5 mg/gm/year (range -3.7 to +9.3), +1.6 mg/gm/year (range -9.2 to +10.9), and -5.7 mg/gm/year (range -12.0 to +0.2) in patients receiving ST, PME, and RCE respectively ( $p = .0235$  using Kruskal Wallis test). Alloimmunization rates were 0.51/100 units and 0.50/100 units for ST/PME and RCE respectively ( $p = .78$ ).

**Conclusion:** When used in conjunction with iron chelation, chronic RCE is highly effective at reducing iron overload while maintaining HbS 30-35%, and does not adversely affect alloimmunization rates.

Platform Session #4009

## **COPY NUMBER NEUTRAL LOSS OF HETEROZYGOSITY OF CHROMOSOME ARM 6p IN ACQUIRED APLASTIC ANEMIA: FREQUENCY, CLONAL EVOLUTION AND CLINICAL SIGNIFICANCE**

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**Background:** Clonal hematopoiesis in patients with Acquired Aplastic Anemia (aAA) may arise in response to selective pressure caused by immune-mediated hematopoietic stem/progenitor cell destruction. Acquired copy-number neutral loss of heterozygosity on the short arm of chromosome 6 (6p CN-LOH) is a recurrent clonal abnormality in aAA. The HLA loci map to 6p, thus 6p CN-LOH may allow for immune escape and restoration of hematopoiesis through the



deletion of HLA alleles targeted by auto-reactive T cells.

**Objectives:** To investigate the frequency, evolution and clinical significance of 6p CN-LOH in our institutional aAA cohort.

**Design/Method:** Bone marrow or peripheral blood samples from patients with aAA and related conditions (paroxysmal nocturnal hemoglobinuria or hypocellular myelodysplastic syndrome) were analyzed for 6p CN-LOH by genome-wide single nucleotide polymorphism arrays (SNP-A). High resolution HLA typing and next generation sequencing (NGS) of HLA loci were used to define allele frequencies within our cohort and identify specific alleles that were functionally deleted within 6p CN-LOH clones.

**Results:** Seventy-five subjects were included (median age 14, range 0.6-67 years); 95% had a SNP-A performed. The prevalence of 6p CN-LOH was 11.3% (n=8). Of the six patients with 6p CN-LOH who received first-line immunosuppressive therapy (IST), none achieved a complete response (failed/relapse n=3, partial response n=3). In patients with follow-up SNP-A data, 6p CN-LOH clones remained mostly stable in terms of size and distribution. No patient with 6p CN-LOH developed MDS-defining cytogenetic abnormalities. Several HLA alleles were over-represented in our aAA cohort compared to ethnicity-matched controls. NGS enabled identification of specific HLA alleles lost in large 6p CN-LOH clones. Two patients had evidence of oligoclonal loss of HLA alleles by two different mechanisms: CN-LOH and loss-of-function mutations, consistent with a selective survival advantage for clones lacking these alleles.

**Conclusion:** 6p CN-LOH is one of the most common clonal abnormalities in aAA, and does not appear to predispose to MDS. Future studies are needed to confirm our finding that aAA patients with 6p CN-LOH clones may exhibit poor responses to IST. Further molecular investigations into clonal changes affecting the HLA locus may provide critical insights into the immune pathogenesis of aAA.

Platform Session #4010

## **EXTENDED GENETIC TESTING OF ALPS AND ALPS LIKE DISORDERS REVEALS NOVEL DISORDERS OF IMMUNE DYSFUNCTION, INCLUDING DEFECTS IN CTLA4, PI3KCD, AND STAT3 GENES**

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**Background:** The understanding of basic human biology has benefited through study of rare diseases. We sought to evaluate patients referred to our institution and meeting the diagnostic criteria for ALPS and ALPS-like disorders of the immune system with undetermined genetic defects (ALPS-U) in order to discover the cause and prognosis of their disease, as well as explore the possibility of more targeted treatments.

**Objectives:** To identify novel disorders of immune dysfunction by studying a cohort of patients with unique ALPS-like clinical phenotypes through extended genetic testing.

**Design/Method:** 36 patients (Males 30, Females 6) with a median age at presentation of 5 years (range birth-23 years) with lymphadenopathy, splenomegaly (86%) and multilineage cytopenias (AIHA and ITP 81%, AIN 53%) were included in this cohort. Eight patients were receiving IVIG supplementation for hypogammaglobulinemia and 4 patients had a lymphoid malignancy. Unique extranodal inflammatory and infiltrative lymphoid lesions were noted for 21 patients in

the following organs (n): lungs (21), liver (9), gut (8), brain (2), CSF pleocytosis (8) and pericarditis (2). Targeted candidate gene sequencing based on specific clinical phenotypes as well as panel-based and Whole Exome Sequencing (Agilent capture with 50-100x Illumina sequencing) were utilized.

**Results:** Genetic diagnoses could be ascertained in 33% (12/36) of the patients. History of lung, brain and gut lesions were notable in patients with mutations in *CTLA4* and related *LRBA* genes (n=5). Two patients with RAS-Associated Leucoproliferative Disorder due to *KRAS* mutations had pericarditis. Three patients with *STAT3* gain-of-function mutations were also identified, two of them had pulmonary infiltrates and one of them had CSF pleocytosis with cerebellar atrophy. Recurrent sinopulmonary infections were associated of PASLI disease due to *PIK3CD* mutations. Detailed clinical presentations and treatment approaches for patients with *CTLA4* (n=14) and *PIK3CD* (n=36) mutations seen at our center will be discussed.

**Conclusion:** It is critical to foster multidisciplinary collaborative clinical research across geographic boundaries to undertake diligent long-term follow up of patient cohorts with unique clinical phenotypes. This will enable us to evaluate the natural history and treat many novel human genetic disorders appropriately in light of rapidly evolving genomic technologies.

Young Investigator Award Winner #4011

## THE CRM1 NUCLEAR EXPORT RECEPTOR ACTIVATES HOXA GENE EXPRESSION IN SEVERAL LEUKEMIA MODELS

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**Background:** *HOXA* genes are effectors of oncogenic transformation that are frequently upregulated in pediatric acute leukemias. The *CALM-AF10* chromosomal translocation is associated with *HOXA* gene overexpression. We have previously shown that a nuclear export signal within *CALM* interacts with the CRM1 nuclear export receptor and that the CRM1/*CALM-AF10* interaction is essential for *HOXA* gene upregulation and leukemia development in mice.

**Objectives:** To determine whether CRM1 participates in *HOXA* upregulation in MLL- and NUP98-fusion associated leukemias, and whether CRM1 can substitute for *CALM* in leukemogenic fusions.

**Design/Method:** We measured *Hoxa* transcript levels in murine leukemia cells treated with the CRM1 inhibitor Leptomycin B (LMB). We created artificial retroviral *CRM1* fusion constructs and assessed their leukemogenic potential *in vitro* and *in vivo*.

**Results:** We found that brief LMB treatment of *MLL-AF10*, *MLL-ENL* or *NUP98-AF10* leukemia cells causes a 50% reduction of *Hoxa7*, *Hoxa9* and *Hoxa10* levels, similar to that observed in *CALM-AF10* leukemia cells. In an *in vitro* murine bone marrow (BM) clonogenic assay, native CRM1 overexpression did not transform, while *CRM1-AF10* or *CRM1-ENL* fusions resulted in immortalization. To investigate leukemogenic potential *in vivo*, we transplanted mice with retrovirally transduced BM progenitors. *CRM1-AF10* induced myeloid neoplasms with a low penetrance and long latency (7/20 mice developed leukemias between 160-350 days). In contrast, *CRM1-ENL* rapidly induced acute myeloid leukemias in 8/9 recipients, between 59-140 days. These primary leukemias were transplantable, causing leukemias with a shorter latency. Both *CRM1-AF10* and *CRM1-ENL* leukemia blasts expressed elevated levels of *Hoxa* genes.

**Conclusion:** Our results demonstrate that CRM1 regulates the expression of *Hoxa* genes in

murine leukemia cells, and that CRM1 fusions can drive murine leukemogenesis. Of note, a *CRM1-AF10* fusion gene was recently identified in a T-ALL patient (*BLOOD*, 2014). Our data also suggest that the efficacy of CRM1 inhibitors currently undergoing clinical trials, could be mediated in part by their ability to block CRM1-dependent transcriptional activation of *HOXA* genes.

Young Investigator Award Winner #4012

## **RNA NANOPARTICLE VACCINES FACILITATE AND SUSTAIN ADOPTIVE CELLULAR THERAPY TARGETING PEDIATRIC INTRACRANIAL MALIGNANCIES**

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**Background:** Pediatric brain tumors are the number one cause of solid cancer death in children, and thus necessitate the development of novel targeted therapeutics. Adoptive cellular therapy (ACT) can target tumor associated antigens with exquisite specificity and has shown promise through chimeric antigen receptor (CAR) transfection or dendritic cell (DC) priming of patient specific T lymphocytes. However, these platforms have been limited by inadequate T cell persistence and by their inherent cost and complexity. To circumvent these challenges, we have developed novel "off the shelf" lipophilic RNA nanoparticles (RNA-NPs) that can be used as cancer vaccines to transfect antigen presenting cells (APCs) *in vivo* thereby inducing anti-tumor immunity against intracranial tumors in pre-clinical ACT model systems.

**Objectives:** We sought to access if vaccination with amplified tumor derived RNA encapsulated in lipophilic NPs could transfect APCs *in vivo*, induce anti-tumor immunity and sustain ACT in pre-clinical high grade glioma (HGG) and medulloblastoma (MB) models.

**Design/Method:** We studied the *in vivo* immunogenicity and anti-tumor efficacy of DS Red labeled *ex vivo* activated tumor specific T cells given concurrently with RNA-NPs in HGG and MB bearing mice in ACT model systems.

**Results:** We screened commercially available and clinically translatable NP formulations and determined that the cationic liposome DOTAP was the most superior NP for delivery of RNA to APCs *in vitro* and *in vivo*. Afterwards, we verified that these particles preserve RNA stability over time, and retain the capacity to induce *in vivo* gene expression. These RNA-NPs induced potent T cell immunity; superior to peptide vaccines formulated in Complete Freund's Adjuvant (CFA) and could be further enhanced through incorporation of immunomodulatory RNAs such as GM-CSF. Finally, we demonstrated that RNA-NPs induce anti-tumor efficacy sustaining ACT in murine HGG and MB models.

**Conclusion:** Clinically translatable NPs complex tumor derived RNA and sustain T cell proliferation and persistence *in vivo* circumventing many of the complexities inherent to current ACT models. These vaccines represent a novel platform for inducing potent nontoxic immunity against intracranial tumors that can be harnessed to provide a more effective and specific therapy critical in improving clinical outcomes for children affected by these malignancies.

Platform Session #4013

## **CLINICAL AND BIOLOGIC PREDICTORS OF MYCN AMPLIFICATION IN**

## NEUROBLASTOMA: A REPORT FROM THE INTERNATIONAL NEUROBLASTOMA RISK GROUP (INRG) PROJECT

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**Background:** *MYCN* amplification is a hallmark of aggressive neuroblastoma and is one of the strongest independent adverse prognostic factors in this disease. However, a comprehensive analysis of the predictors of *MYCN* amplification itself has not been reported.

**Objectives:** To identify groups of clinical and/or biologic factors that distinguish patients with a high likelihood of having *MYCN* gene amplification from those having a low likelihood.

**Design/Method:** INRG data were analyzed from 7,102 patients diagnosed with neuroblastoma from 1990-2002 with known *MYCN* status. Descriptive statistics and chi-squared tests were used to analyze univariate clinical (age at diagnosis, ferritin and LDH level, INSS stage, primary tumor site) and biologic (DNA ploidy, MKI, tumor differentiation, segmental chromosomal aberrations) variables. Univariate logistic regression was used to identify variables that were most highly predictive of *MYCN* amplification status. Manual multivariate recursive partitioning was used to identify subgroups of patients with maximal difference in rates of *MYCN* amplification.

**Results:** 7,102 / 8,800 patients had known *MYCN* status. All clinical and biologic variables were statistically significant univariate predictors of *MYCN* amplification. The odds of *MYCN* amplification were 8.4 fold greater for high LDH (vs. low LDH), and the odds were 19.8 greater for 1p deletion (vs. normal 1p). Recursive partitioning that incorporated both clinical and biological variables identified subgroups of patients with disparate rates of *MYCN* amplification. The most extreme subgroups were: Patients with high LDH who had adrenal primary tumors that were poorly differentiated and harbored a chromosome 1p deletion (85.7% had *MYCN* amplification) compared to those with localized tumors with hyperdiploidy, low MKI, and lacking chromosome 1p aberration (0.6% had *MYCN* amplification). Age and ferritin level were not significant predictors of *MYCN* status in this model.

**Conclusion:** Recursive partitioning identifies subgroups of neuroblastoma patients who have highly disparate rates of *MYCN* amplification. These findings can be used to inform investigations of molecular mechanisms of *MYCN* amplification and support the importance of having tumor tissue available at diagnosis to provide biological data that may inform diagnosis, prognosis, and selection of therapy.

Platform Session #4014

## NORADRENALINE TRANSPORTER GENE TRANSFER VIA ONCOLYTIC VIROTHERAPY ENHANCES 131I-mIBG UPTAKE: A NOVEL TARGETED APPROACH TO HIGH-RISK NEUROBLASTOMA

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**Background:** Outcomes for children with recurrent/refractory neuroblastoma remain dismal and novel targeted therapies are needed. Increasing NAT (noradrenaline transporter) expression to

enhance  $^{131}\text{I}$ -mIBG therapy is one investigational approach as *MYCN* amplified tumors are associated with lower NAT expression.<sup>1</sup> HSV1716 is an oncolytic herpes virus genetically altered to selectively replicate in cancer cells that is currently in a pediatric phase 1 clinical trial. HSV1716/NAT is a derivative that was constructed to deliver the NAT cDNA to cancer cells. Previous studies have shown increased antitumor effects in glioma and melanoma xenografts exposed to  $^{131}\text{I}$ -mIBG and HSV1716/NAT.<sup>2</sup>

**Objectives:** Determine the antitumor efficacy of the oncolytic virus HSV1716/NAT in combination with  $^{131}\text{I}$ -mIBG in neuroblastoma cell lines.

**Design/Method:** PPTP database mining and qRT-PCR were used to determine endogenous NAT expression in 12 neuroblastoma cell lines and in xenograft tumors grown in nude (nu/nu) mice. The cell lines' response to HSV1716/NAT were explored via gene transfer, viral production, and cytotoxicity assays. Neuroblastoma cells were exposed to HSV1716 and/or  $^{131}\text{I}$ -mIBG to determine cytotoxicity and  $^{131}\text{I}$ -mIBG uptake.

**Results:** Variation in endogenous NAT mRNA expression exists among neuroblastoma cell lines and in xenograft models. We found variations in HSV1716/NAT susceptibility and permissivity among the neuroblastoma cell lines in addition to effective NAT production. Increased cytotoxicity was noted with the combination of virus and  $^{131}\text{I}$ -mIBG than with either treatment alone. Neuroblastoma cells infected with HSV1716/NAT have increased NAT mRNA expression, increased specific uptake of  $^{131}\text{I}$ -mIBG via NAT, and increased cytotoxicity with  $^{131}\text{I}$ -mIBG.

**Conclusion:**  $^{131}\text{I}$ -mIBG uptake and neuroblastoma cytotoxicity increase when treated with both HSV1716/NAT and  $^{131}\text{I}$ -mIBG. Further studies will determine if the expected increase in cytotoxicity is due to a radiation-induced bystander effect or increased  $^{131}\text{I}$ -mIBG uptake. Additionally, studies are underway to evaluate the anti-tumor effects and animal survival in mice with neuroblastoma xenografts exposed to HSV1716/NAT,  $^{131}\text{I}$ -mIBG, and the combination of oHSV and  $^{131}\text{I}$ -mIBG. We aim to translate this project into a clinical trial with potential to expand to other pediatric tumor types.

1. DuBois, Int J Mol Imaging, 2012
2. Sorensen, J Nucl Med, 2012

Platform Session #4015

## MULTISPECIFIC T CELLS FOR THE TREATMENT OF HIGH GRADE GLIOMAS

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**Background:** High grade gliomas (HGG) carry a dismal prognosis. Our group has shown that patients' HER2-specific chimeric antigen receptor (CAR) T-cells kill autologous HGG, yet 40% of tumors recur in experimental animals. Recurrences are composed of HER2 negative escape variant cells that maintain positivity for two other glioma antigens, IL13R $\alpha$ 2 and EphA2. We hypothesized that co-targeting of these escape variants will enhance the anti-glioma activity of CAR T cells.

**Objectives:** The intent of this project is to develop a multi-specific T-cell product for the adoptive immunotherapy of HGG.

**Design/Method:** We generated and tested the effector functions of patients' T-cells co-

expressing CARs for HER2, IL13R $\alpha$ 2 and EphA2 against autologous HGG cells derived from surgical excision samples.

**Results:** Primary patient HGG samples exhibited varied expression of the three target antigens, HER2, IL13R $\alpha$ 2 and EphA2. In order to render single patients' T cells tri-specific, we designed a transgene incorporating 3 encoding regions for HER2, IL13R $\alpha$ 2 and EphA2 CARs separated by 2A sequences in a single cassette driven by a CMV promoter. We used a retroviral system to stably integrate this transgene into the primary T cell genome. CAR-specific flowcytometry indicated proportionate stable expression of individual CAR molecules on the T cell surface. These T cells show distinct specificity for each of the three glioma antigens evidenced by activation, proliferation, and cytolytic function in immunoassays.

**Conclusion:** The heterogeneity of antigenic expression in HGG and antigen escape justify targeting multiple glioma antigens simultaneously. We have successfully generated and tested a multi-specific T cell product for adoptive transfer that could offset antigen escape and exhibit an enhanced anti-glioma efficacy.

Platform Session #4016

## **PEDIATRIC PERSONALIZED ONCOGENOMICS (PEDSPOG) -INITIAL OUTCOMES**

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**Background:** Children with relapsed solid malignancies have limited therapeutic options. Advancements in genomic technologies have resulted in the ability to use real-time DNA and RNA sequencing in an attempt to identify individualized therapeutic targets.

**Objectives:** Utilization of integrative genomic information in order to augment chemotherapy decision making in children with relapsed or refractory tumors.

**Design/Method:** Children with relapsed or refractory solid tumors were identified by their treating oncologist. After informed consent a tumor biopsy was performed and whole genome and transcriptome sequencing was completed. Sequencing was also performed on peripheral blood for germline testing and on the archived original primary tumors (fresh frozen tissue or FFPE) to examine changes in the cancer over time. Each patient's tumor genomic data was compared to the literature and to a comprehensive drug database with the aim of identifying available therapies most likely to target individual tumors based on the presence of somatic mutations or aberrantly expressed genes. All cases were reviewed at a weekly tumor board attended by genomic scientists, pathologists, and clinicians and treatment options were discussed. Therapies were ultimately decided upon by the treating oncologist.

**Results:** Fourteen subjects have been approached and 12 subjects have enrolled (four with neuroblastoma and one each with CNS sarcoma, infantile fibrosarcoma, plexiform neurofibroma, osteosarcoma, lymphoma, angiosarcoma, metastatic melanoma and fibrovascular tumor NYD). In 9 of 12 subjects sufficient viable tumor tissue for sequencing was obtained from a minimally invasive biopsy (one required a second biopsy) and results are available for 7 (2 cases pending). The median turnaround time was 6 weeks. In 6 of 7 subjects informative genomic findings were identified and in 5 subjects options for targeted therapies were identified. Three subjects were started on therapies informed by results of PedsPOG with a further subject awaiting measurable disease to reappear prior to starting therapy. Clinical improvement was seen

in all three (two treated with crizotinib, one on ruxolitinib) with one radiological partial response and stable disease in two.

**Conclusion:** The initial pilot project into pediatric personalized oncogenomics has yielded interesting findings and therapeutic options in a significant proportion of those enrolled. Ongoing cases are being recruited into the study.

Platform Session #4017

## **SEXUAL DYSFUNCTION IN YOUNG ADULT SURVIVORS OF CHILDHOOD CANCER**

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**Background:** Disruption of psychosexual development and sexual dysfunction are ranked among the most profoundly distressing long-term side effects of pediatric cancer treatment yet sexual dysfunction in young adult survivors of childhood cancer (YASCC) is not well understood. Prior research revealed that nearly 50% struggled with at least one major sexual problem, and 30% reported two or more problems. Long-term negative implications of unmanaged sexual dysfunction include threats to adult identity, intimacy and low self-esteem, therefore emphasizing a need to effectively address this issue in YASCC.

**Objectives:** To better characterize sexual dysfunction and to identify potential causal factors in YASCC.

**Design/Method:** Semi-structured interviews were held with 17 YASCC (ages 18-32 years, 7 men, 10 women) reporting sexual dysfunction. Interviews were conducted in English by phone or in person. The interview guide was developed based on literature review of sexual dysfunction in childhood cancer survivors, sexual dysfunction in adult survivors of adult cancers, and adolescent/young adult sexual development. The interview transcript underwent pre-testing with YASCC. All interviews were audio-recorded, transcribed verbatim and analyzed using accepted qualitative methods aided by Nvivo10 analysis software.

**Results:** Common sexual function problems reported by participants included difficulty relaxing and enjoying sex and difficulties with arousal/achieving orgasm. Few participants reported physical challenges, such as fatigue, interfering with sexual function. All but 2 participants reported strong interest in engaging sexual activity. Themes from coded transcripts included significant disruption of social life due to cancer diagnosis and treatment, negative body image resultant of cancer and treatment side effects, concerns about missed opportunities for fertility preservation/future fertility, concerns about disclosure of cancer history, and poor communication with oncologists regarding sexual function. Participants related these concerns to their histories of childhood cancer. The majority of participants felt pediatric oncologists should discuss sexual health with patients.

**Conclusion:** The experiences described by YASCC provide valuable insight into the nature and potential etiologies of sexual dysfunction in this population. This data sets the groundwork for future research on sexual dysfunction screening measures, patient-physician communication of developmentally critical issues such as sexual health, and appropriate and effective interventions to address sexual dysfunction in YASCC.

## INTERIM ANALYSIS OF THE ASPHO MENTORING PROGRAM

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**Background:** Effective networking and mentorship are critical determinants of academic success. While mentoring programs already exist at the institutional and professional society level, these are often focused programs that fail to meet the full spectrum of mentoring needs for junior faculty and fellows. The Early Career Subcommittee of the ASPHO Professional Development Committee designed and implemented a novel mentoring program in 2013 that allows mentees to self-select their mentors from an internet-based list of mentor profiles.

**Design/Method:** Junior faculty or current trainees were allowed to self-select a mentor from the list of mentor profiles posted on the ASPHO website, and mentee-mentor pair worked together to define written goals and plans for achieving their objectives. Outcomes and productivity of each mentee-mentor pair were determined using a semi-annual survey tool.

**Results:** Thirty-three mentees and thirty-five mentors have been enrolled in the program, and twenty-six mentee-mentor pairs have been formed. Eighteen pairs were eligible to participate in the semi-annual outcome survey, and responses were received from seventeen (94%) mentors and sixteen (89%) mentees. The majority of mentors (83%) and mentees (81%) achieved some of their goals within this six-twelve month period. Pairs communicated every 11 weeks on average (range 2-24 weeks) via phone (83%), e-mail (72%), and in-person at the annual ASPHO meeting (64%). About 82% of mentors and 93% of mentees are satisfied with the structure of their mentoring relationship. Moreover, the majority of mentors (77%) and mentees (87%) considered their experiences to be rewarding. All mentees and 89% of mentors planned to continue their mentoring relationship. We also collected data on barriers and facilitators of the mentoring relationship as well as suggestions for further program improvement.

**Conclusion:** The ASPHO Mentoring Program provides an opportunity to meet the diverse professional development needs of a broad spectrum of ASPHO junior faculty and fellow members. This interim analysis of the mentorship program suggests a continued clear benefit from career development guidance for Early Career ASPHO members. New mentees and mentors are encouraged to participate in the ASPHO Mentoring Program.

## HEMO-MILESTONES TOOL INCREASES ASSESSMENT OF SELF-MANAGEMENT COMPETENCY AND PLAN FOR SKILL BUILDING IN PATIENTS WITH HEMOPHILIA

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**Background:** Longitudinal attainment of self-management skills, disease knowledge, and a documented transition plan are key drivers for transfer from pediatric to adult subspecialty care



for patients with chronic disease. Our hemophilia center had no standardized approach for assessing developmentally appropriate patient competencies or independence readiness. Retrospective review of comprehensive clinic visits, May-July 2014 (N=29), revealed that only 21% of patients had an overall assessment of hemophilia competency documented and 55% had a plan for transition or skill building.

**Objective:** (SMART aim) To achieve a sustained rate of 90% of hemophilia patients with uniform documentation of assessment of disease competency and skill building plan at their comprehensive visit within 3 months of tool implementation. Secondary SMART aim, to identify specific competencies with >20% deficiency rate.

**Design/Method:** Using institutional transition tools, hemophilia age-specific guidance documents, and clinical experience we created a *HEMO-milestones tool* comprised of four core hemophilia competencies and associated age-specific milestones. Barriers to tool implementation and inclusion in the patient medical record and age appropriateness of milestones were explored and modified in four PDSA ramps.

**Results:** Thirty one patients, age 2-21 years, were seen in hemophilia comprehensive clinic between 9/15/14 and 12/15/14. 97% had an overall competency assessment and 93% had progress plans completed. 29 patients (93%) were evaluated for each of the 4 competencies. Six patients (19%) were below expectation for age in at least one competency and 10% in  $\geq 2$  competencies. Age range for patients with a deficiency was 11-18 years old. Achievement in the communication competency, which addresses the patient's ability to interact with providers and access medical care, was the most challenging for patients; five patients (16%) were below expectation. Time from visit to tool documentation completion decreased from 49 days to 4 days.

**Conclusion:** This tool allows standardized hemophilia competency assessment and skill building, supporting a longitudinal approach to patient independence. Routine use will provide data on success rates for age-specific milestones for hemophilia patients and inform patient-targeted education and topics for bi-annual center education events. Longitudinal attention to patient competency and skill should facilitate medical independence and transition of care.

Platform Session #4020

## THE NORTH AMERICAN SHWACHMAN-DIAMOND SYNDROME REGISTRY: Five YEARS OF FOLLOW-UP

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**Background/Objectives:** The North American Shwachman-Diamond Syndrome Registry (SDSR) opened in December 2008 with the goal of understanding the natural history of Shwachman-Diamond Syndrome (SDS) to improve medical management and treatment.

**Design/Method:** The SDSR enrolls patients with biallelic *SBDS* gene mutations or with clinical features of SDS including exocrine pancreatic dysfunction in the presence of bone marrow failure.

**Results:** One hundred twenty five patients have been enrolled and data are available for 83 patients. Study subject ages span 2-52.4 years with a median age of 11.1 years. Fifty-five individuals are genetically defined with biallelic *SBDS* mutations, while 29 individuals lack biallelic *SBDS* mutations. Four patients are deceased. Two of the deceased patients were *SBDS*

mutation-positive and passed away in their early 20s. Ongoing characterization of *SBDS* mutation-negative individuals has identified subgroups of clinically defined SDS individuals, as well as a more heterogeneous subgroup with features of SDS but in whom a clinical diagnosis cannot be confirmed by classic diagnostic criteria. Cytopenias were universal, with intermittent neutropenia the most frequent. Fourteen patients have required use of G-CSF. Eleven mutation-negative patients had normal pancreatic imaging studies while 1 had pancreatic lipomatosis. Two individuals developed acute myeloid leukemia: one mutation-positive and one for whom mutation status was unknown. None of the mutation-negative patients have undergone hematopoietic stem cell transplant (HSCT) to date. Eight *SBDS* mutation-positive patients underwent HSCT, while mutation status was unknown for 2 patients. Data from the SDSR continues to identify clinical features associated with SDS including congenital anomalies and endocrine abnormalities. Nineteen individuals have survived into adulthood with persistence of clinical symptomatology including both pancreatic, neuropsychological, skeletal dysfunction, and hematologic disease and progression of cytopenias or dysplasia in a subset.

**Conclusion:** These data have expanded our understanding of the disease phenotype, and identified a broader range of clinical presentations for SDS altering the clinical approach to diagnosis and management. The SDSR continues to grow and mature as a resource for clinical and biological studies in the study of this rare disorder stimulating further progress in our understanding of genetic etiology, mechanism, and disease pathophysiology and treatment.

Platform Session #4021

## THE GENOMIC LANDSCAPE OF PEDIATRIC ACUTE MEGAKARYOBLASTIC LEUKEMIA

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**Background:** Non-Down syndrome acute megakaryoblastic leukemia (non-DS-AMKL) carries a dismal prognosis with overall survival of 14-34%. Aside from the t(1;22)(p13;q13) seen in infants, the genomic landscape of non-DS-AMKL has remained elusive. Recently, Gruber et al. identified a cryptic inversion on chromosome 16 fusing *CBFA2T3* to *GLIS2* in up to 27% of cases by RNAseq in a discovery cohort of 14 patients.

**Objectives:** To more fully understand the leukemic drivers in this rare subtype of myeloid leukemia, we have extended our analysis of non-DS-AMKL through next generation sequencing of an additional 130 cases (105 pediatric and 25 adult) in addition to our initial discovery cohort.

**Design/Method:** A total of 144 non-DS-AMKL cases (25 adult, 119 pediatric) will undergo RNA sequencing to evaluate for the presence of gene fusion events and/or whole exome sequencing for the detection of single nucleotide variants, insertions, and deletions. Gene fusions will be validated using PCR followed by Sanger sequencing and mutations will be validated by targeted capture.

**Results:** To date 92 patient samples have been RNA sequenced and analyzed. Whereas the majority of pediatric cases (77%) contained an identifiable gene fusion event, 12 of 25 adult cases (48%) lacked any detectable fusion. Recurrent fusions identified in pediatric cases include

*CBFA2T3-GLIS2* (17.2%), *MLL* rearrangements (*MLLr*; 13.8%), *HOX* rearrangements (*HOXr*; 13.8%), *NUP98-KDM5A* (10.3%), and *RBM15-MKL1* (9.2%).

Single nucleotide variations (SNVs) and insertions/deletions (indels) have been catalogued for 109 patients that are undergoing validation. Recurrently mutated genes include *GATA1*, *MPL*, *JAK* kinases, *TP53*, and *NOTCH* and *RAS* pathway genes, which have all been previously reported in AMKL.

**Conclusion:** Pediatric non-DS-AMKL is a heterogeneous disease characterized largely by the presence of recurrent gene fusion events. Despite this heterogeneity, patients can be grouped into those that carry fusion events leading to HOX mediated leukemogenesis (*MLLr*, *NUP98-KDM5A*, *HOXr*) and those that do not (*CBFA2T3-GLIS2*, *RBM15-MKL1*). Patients not carrying a fusion gene harbored either a *GATA1* truncating mutation, or multiple pathologic SNVs in genes previously demonstrated to play a role in acute myeloid leukemia.

## References:

Gruber et al., Cancer Cell 22:683-697, 2012

Platform Session #4022

## MATRIX METALLOPROTEINASE-14 INHIBITION AS A NOVEL THERAPEUTIC APPROACH FOR B-ALL AND AML: POISONING THE LEUKEMIA NICHE

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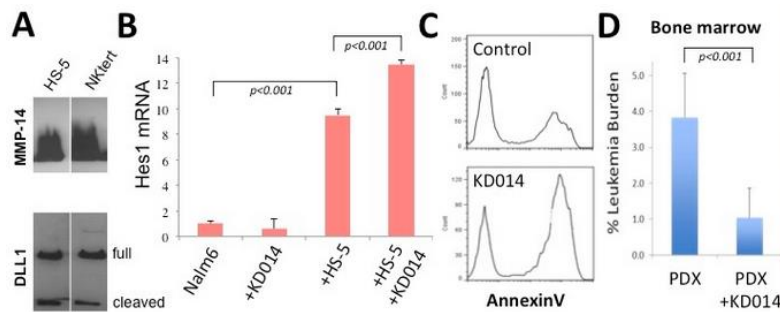
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**Background:** Activation of Notch signaling in B-ALL and AML cells induces growth arrest and apoptosis. Importantly although the Notch ligand DLL1 is expressed in bone marrow stroma it was recently discovered that matrix metalloproteinase-14 (MMP-14) cleaves DLL1, leading to decreased Notch signaling in hematopoietic cells. Thus, we hypothesized that blocking MMP-14 would increase DLL1 in the bone marrow and thus inhibit growth and survival of B-ALL and AML via Notch signaling.

**Objective/Method:** Using a panel of human B-ALL and AML leukemia lines and human bone marrow stromal lines expressing DLL1, we tested the effects of inhibiting MMP-14 on leukemia survival *in vitro* and *in vivo*.

**Results:** MMP-14 protein was strongly expressed in stromal cells, and DLL1 was present as a full-length surface protein, but also the N-terminally cleaved version (panel A). Addition of MMP-14 specific inhibitory antibody (KD014) increased the presence of DLL1 on the surface of stromal cells, increased the level of Notch signaling in leukemia cells (>40%, qRT-PCR of HES1, panel B), and increased the rate of co-culture-associated apoptosis by >50% (panel C). Similarly, *in vivo* KD014 treatment of mice engrafted with a human B-ALL patient derived xenograft led to 4-fold lower leukemia burden in the bone marrow (panel D).

**Conclusion:** We reveal that inhibition of MMP-14 is a novel therapeutic approach for B-ALL and AML. This occurs through a novel host-based mechanism where enhanced expression of Notch ligand DLL1 in the bone marrow stroma “poisons” the bone marrow for B-ALL and AML by inducing Notch-mediated apoptosis.



Platform Session # 4023

## MRX2843, A NOVEL DUAL MERTK & FLT3 INHIBITOR WITH ACTIVITY AGAINST RESISTANCE-CONFERRING FLT3 MUTATIONS IN ACUTE MYELOID LEUKEMIA

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**Background:** FLT3 tyrosine kinase internal tandem duplication mutations (FLT3-ITD) are present in 15-30% of acute myeloid leukemia (AML) and portend a poor prognosis. Although initial responses to current FLT3 inhibitors have been observed, patients develop resistance via kinase domain point mutations at amino acid F691 or D835.

Mer receptor tyrosine kinase (TK) is overexpressed in 80-100% of AML patient samples, and shRNA-mediated inhibition demonstrated anti-leukemic effects in AML.

**Objectives:** We describe pre-clinical activity of MRX2843, a novel MerTK and FLT3 targeted small molecule inhibitor with activity against AC220-resistant FLT3 point mutations.

**Design/Method:** AML cell lines Kasumi-1 (FLT3-WT/MerTK<sup>+</sup>) and Molm14 (FLT3-ITD/MerTK<sup>-</sup>) were treated with 25-300nM MRX2843. Inhibition of FLT3 and MerTK activation and downstream signaling was assessed via immunoblot. Induction of apoptosis was determined by flow cytometry after staining with YO-PRO-1 iodide and propidium iodide.

Two derivatives of Molm14 harboring either D835Y (Molm14:D835Y) or F691L (Molm14:F691L) FLT3 point mutations were used to test the activity of MRX2843.

Mouse xenografts were established by intravenous injection of cell lines into NSG mice. Daily treatment with vehicle, 10mg/kg AC220, or 50mg/kg MRX2843 was administered by oral gavage.

**Results:** Treatment of cell lines with MRX2843 abrogated activation of FLT3 and MerTK as well as downstream signaling pathways, including STAT5, AKT and ERK1/2. Treatment led to significant induction of apoptosis compared to vehicle-treated controls in both Kasumi-1 (57±5% versus 7±2%, p<0.001) and Molm14 (84±2% versus 5±2%, p<0.001), as well as mutant cell lines Molm14:D835Y (80±7% versus 10±1%, p<0.001) and Molm14:F691L (61±16% versus 12±1%, p<0.001). In contrast, both mutant cell lines were resistant to treatment with AC220. In vivo, MRX2843 prolonged survival of NSG mice transplanted with Molm14:D835Y cells compared to AC220 or vehicle (median survival 71, 35, and 20 days, respectively, p<0.01). Similarly, survival was prolonged in mice injected with Molm14:F691L cells when treated with MRX2843 (median survival of 87 days) versus AC220 (57 days) and vehicle (45 days, p<0.01).

**Conclusion:** MRX2843 is a novel MerTK and FLT3 inhibitor that retains activity against clinically relevant, resistance-conferring FLT3 point mutations and prolongs survival in murine models of AML. These data support the use of MRX2843 in early phase clinical trials.

Platform Session #4024

## **MAP2K1 MUTATIONS IN LANGERHANS CELL HISTIOCYTOSIS (LCH) AND JUVENILE XANTHOGRANULOMA (JXG) RESULT IN CONSTITUTIVE ACTIVATION OF MEK AND RESPOND TO MEK INHIBITORS**

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**Background:** Although most patients with LCH respond to current chemotherapies, many of them relapse. Effective targeted therapies for LCH have been unavailable because of lack of knowledge of the underlying pathophysiology. Previous studies revealed that ~55% of LCH cases carry the *BRAF* V600E mutation, suggesting that activation of the RAF/MEK/ERK pathway plays a role in disease pathogenesis. By targeted sequencing, we identified mutations in *MAP2K1*, the gene encoding MEK, in *BRAF* wild-type LCH and also in JXG. Similar findings were recently reported by other groups in addition to recurring cooperating mutations in non-histiocytic disorders, though these have not yet been found in LCH and JXG.

**Objectives:** We aimed to characterize the spectrum of *MAP2K1* mutations and determine whether cooperating mutations co-existed in *MAP2K1*-mutant LCH. We also sought to determine the impact of the identified *MAP2K1* mutations and test the efficacy of MEK inhibitors.

**Design/Method:** Murine fibroblasts were transduced with wild type or mutant *MAP2K1* (F53\_Q58delinsL, Q58\_E62del), or *BRAF* V600E using retroviral vectors. Using these cells, we compared available MEK inhibitors AZD-6244 and PD-0325901, the *BRAF*-inhibitor Vemurafenib, and the ERK inhibitor GDC-0994 in their abilities to block the activation of ERK by these activating mutations.

**Results:** Immunoblotting and flow cytometry assays showed that compared to wild type, mutant *MAP2K1* resulted in greater phosphorylation of downstream ERK, both at baseline and upon activation. We further found that both AZD-6244 and PD-0325901 inhibited ERK phosphorylation induced by mutant *MAP2K1* or *BRAF*-V600E. As expected, Vemurafenib inhibited *BRAF*-V600E but not the *MAP2K1* mutations. Additionally, our sequencing platform identified apparent loss-of-function mutations in *MAP3K14*, *ARID1A*, and *SUZ12*, all of which have been implicated in tumorigenesis.

**Conclusion:** MEK inhibitors currently in clinical development effectively inhibit phospho-ERK signaling and may represent a valid therapeutic option for patients with *MAP2K1*-mutant LCH and JXG. Additionally, disease activity may be modified by cooperating mutations in known oncogenes and tumor suppressors.

Platform Session #4025

## **THROMBIN INDUCED APOPTOSIS IS PROTEASE ACTIVATED RECEPTOR (PAR)-3/-4 DEPENDENT IN HUMAN PODOCYTES AND PAR-1/-4 DEPENDENT IN RAT PODOCYTES**

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**Background:** Nephrotic Syndrome (NS), one of the most common forms of glomerular disease, is characterized by massive proteinuria with injury of specialized glomerular cells called podocytes. There is evidence to support that thrombin generation is enhanced in NS. *In vitro* studies have demonstrated that exposure to high concentrations of thrombin can injure podocytes, suggesting that thrombin may exacerbate glomerular injury.

**Objectives:** Our objective was to determine the molecular mechanisms by which thrombin induces podocyte injury. Thrombin activates other cells via the protease activated receptor (PAR) system. Thus, we hypothesized that thrombin exacerbates glomerular injury by enhancing podocyte apoptosis in a PAR-dependent manner.

**Design/Method:** Experiments were performed with differentiated, conditionally immortalized human and rat podocytes. Podocyte apoptosis was determined after 36 hours of thrombin (20nM) exposure using the TUNEL assay. Specific PAR antibodies and activation peptides (AP) were utilized to determine which PARs mediate thrombin-induced podocyte apoptosis.

**Results:** Thrombin exposure induced a significant increase in apoptosis of human podocytes from 1.8% to 42.9% ( $p<0.05$ ). Blockade of PAR-3 or PAR-4 resulted in a significant decrease in apoptosis [9.2% with hPAR-3 ab and 11.7% with hPAR-4ab] ( $p<0.05$ ). Inhibition of thrombin enzymatic activity with hirudin, a direct thrombin inhibitor, also resulted in a decrease in apoptosis to 2.1% ( $p<0.05$ ). In comparison to a control peptide, only PAR-4 activation peptide significantly increased apoptosis from 1.7% to 40.1% ( $p<0.05$ ). In rat podocytes human thrombin had a similar effect with increased apoptosis from 1% to 33.6% ( $p<0.05$ ). Blockade of PAR-1 and PAR-4 with specific antibodies ameliorated thrombin induced apoptosis [1.9% with rPAR-1 ab and 3.5% with rPAR-4ab] while blockade of PAR-2 and PAR-3 did not have a similar effect ( $p<0.05$ ). Only PAR-1 activation peptide significantly increased rat podocyte apoptosis [37.5%] more than control peptide [13.3%] ( $p<0.05$ ).

**Conclusion:** *In vitro*, thrombin-induced apoptosis appears to be mediated in a PAR-1/-4 dependent manner in rat podocytes but in a PAR-3/-4 manner in human podocytes. Furthermore, these data suggest that thrombin-induced podocyte injury may be mediated in a manner dependent on PAR heterodimerization. Interrupting thrombin-mediated podocyte injury may offer a novel therapeutic approach to reduce podocyte injury associated with nephrotic syndrome.

Platform Session #4026

## **EVALUATION OF ANGIOGENIC CYTOKINES ASSOCIATED WITH THE REACTIVE THROMBOCYTOSIS CAUSED BY IRON DEFICIENCY ANEMIA**

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**Background:** Children with iron deficiency anemia (IDA) can develop reactive thrombocytosis. The processes involved in this phenomenon are unclear. Traditional cytokines involved in megakaryopoiesis such as Thrombopoietin (TPO), IL-6, and IL-11 have not been found to be the cause. Other cytokines have been reported to influence the proliferation and/or

differentiation of megakaryocytes. A recent study observed that VEGFR1-mediated pathway up-regulates CXCR4 on megakaryocytes, causing thrombocytosis by redistribution of megakaryocytes in the bone marrow. Other studies have demonstrated cytokines such as platelet-derived growth factor (PDGF) and FMS-Like Tyrosine Kinase-3 Ligand (FLT-3L) to induce VEGF. These angiogenic cytokines may be responsible for the reactive thrombocytosis associated with IDA.

**Objectives:** To (1) induce IDA in rats through a low iron diet and phlebotomy, and (2) to evaluate if angiogenic cytokines (VEGF, FLT-3L, CXCR4, and PDGF) are associated with reactive thrombocytosis of IDA while confirming that TPO is not involved in this process.

**Design/Method:** Six-week old male Sprague-Dawley rats with jugular vein cannulas were obtained. Diet for control rats (N=9) and iron deficient diet rats (N=18) contained 50 ppm and 7-8 ppm iron in Purina chow respectively. CBC, iron panel, and cytokines were drawn at baseline and five weeks later. Additionally, 1.5 mL of blood was drawn from iron deficient diet rats to further induce anemia. Concentrations of PDGF (platelet-poor plasma), FLT-3L (serum), VEGF (plasma), CXCR4 (plasma), and TPO (plasma) were measured by ELISA. Rats were euthanized by CO<sub>2</sub> asphyxiation and cardiac puncture.

**Results:** IDA with thrombocytosis occurred in iron deficient diet rats at 5 weeks. VEGF, CXCR4, and TPO were not significantly different between groups, but, FLT-3L (p-value <0.0001), and PDGF (p-value <0.0001) were significantly increased at 5 weeks in IDA rats when compared to controls and baseline.

**Conclusion:** We successfully induced IDA in an animal model with coexisting thrombocytosis. Based on our study, this process is independent of TPO, CXCR4, and VEGF. However, we observed an increase in FLT-3L and PDGF. These two angiogenic cytokines may be expanding megakaryocyte progenitors leading to the reactive thrombocytosis seen with IDA. Further studies are required to evaluate the exact mechanism.

Platform Session #4027

## **ERYTHROID CELLS FROM DIAMOND BLACKFAN ANEMIA (DBA) PATIENTS WITH GATA1 AND RIBOSOMAL PROTEIN (RP) MUTATIONS SHOW MARKED DIFFERENCES IN RNA EXPRESSION**

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**Background:** DBA is a rare, congenital bone marrow failure syndrome characterized by severe macrocytic anemia. Approximately 65% of DBA patients have heterozygous mutations in ribosomal protein (RP) genes or rarely mutations in the *GATA1* gene. The mechanism underlying the selectiverethroid failure in DBA is not understood, largely due to the inability to study primary erythroid cells from DBA patients.

**Objectives:** To delineate the mechanisms regulating erythroid differentiation in DBA.

**Design/Method:** CD34+ cells isolated from DBA patient peripheral blood collected prior to transfusion at the DBA Registry were cultured *in vitro*. Protein coding and long non-coding RNA transcripts from primary samples undergoing erythroid differentiation were compared using expressionmicroarray and RNASeq.

**Results:** We characterized DBA patient specimens with mutations in large (*RPL5*) and small (*RPS17*) subunit RP genes, in *GATA1*, and in patients with unknown mutations. At the end of the

culture, we routinely obtained  $6 \times 10^7$  CD235+ erythroid cells from an initial population of  $5 \times 10^4$  normal CD34+ progenitor cells. In contrast to controls, cells from the DBA patients exhibited a significantly reduced growth rate and generated approximately 100-fold fewer CD235+ erythroid cells. We isolated populations of CD41-, CD44+, CD235+ erythroid cells from both control and patient cell cultures and extracted RNA, allowing for the first comparison of mRNA expression in primary DBA erythroid cells. CD235+ cells from the *RPL5* patient showed decreased levels of GAS5 (growth arrest), NOP56 (large subunit formation), and small nucleolar RNAs. The DBA-associated splice donor mutation in *GATA1* results in the exclusive expression of the short form of the GATA1 protein in hemizygous males. Only the short isoform, GATA1s, was expressed in the patient. Northern blot analysis demonstrated that the *GATA1* mutation did not affect pre-ribosomal RNA processing. The microarray and RNASeq expression profiles of the patient differed significantly both from controls and the *RPL5* patient. Known GATA1 target genes were down regulated in the erythroid cells of the patient.

**Conclusion:** DBA patient cells show decreased proliferative and erythroid differentiation capabilities *in vitro*. RNA transcript analysis in DBA patient cells has revealed significant differences between DBA patients with a RP gene mutation and those with a GATA1 mutation. Many GATA1 target genes depend on full length GATA1 for activation.

Platform Session #4028

## **CHEMOTHERAPY-ONLY PREPARATIVE REGIMEN FOR ALTERNATIVE DONOR HEMATOPOIETIC CELL TRANSPLANTATION (HCT) FOR PATIENTS WITH FANCONI ANEMIA (FA): RESULTS OF A MULTI-INSTITUTIONAL STUDY**

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**Background:** HCT for FA remains challenging.

**Objectives:** This multi-institutional study was designed to optimize outcomes of alternative donor HCT in patients with FA without using total body irradiation (TBI). TBI was replaced by busulfan (BU) (to reduce the risk of secondary solid tumors) and BU dose was reduced after first 25 patients to find the lowest acceptable dose.

**Design/Method:** Forty five patients (median age 8.2; range 4.3-44) with history of prior transfusions, androgen use and/or myelodysplastic syndrome were prospectively enrolled (June 2009 - May 2014).

Preparative regimen included: BU 0.8-1.0 mg/Kg/dose IV (first 25 patients) and 0.6-0.8 mg/kg/dose Q 12H (next 20 patients) x 4 doses, cyclophosphamide (10mg/kg), fludarabine (35mg/m<sup>2</sup>) and rabbit ATG (2.5mg/kg) daily x 4 days. BU doses were adjusted based on pharmacokinetics around the first dose. All grafts were T-cell depleted using the CliniMacs CD34 columns (Miltenyi). GvHD prophylaxis was cyclosporine.

**Results:** Transplant outcomes are described in **Table 1**. One patient developed sinusoidal obstruction syndrome of liver early on; none since reducing the BU goal level.

One year probability of overall and disease free survival for the entire cohort was 79.2% (+/- 6.2%) and 76.7 (+/- 6.5%) respectively. OS for patients <10 years of age transplanted for marrow failure was 91.3 % (+/- 5.9%) similar to that reported for historical matched sibling donor HCT outcomes.

**Conclusion:** This chemotherapy only preparative regimen leads to excellent outcomes in



patients undergoing alternative donor HCT for FA, comparable to historical TBI-based protocols, while avoiding short and (to date) long-term toxicities associated with radiation.

Characteristic	Number/Median (range)
Total number of patients engrafted	43*
Days to Neutrophil engraftment	9 (7-15)
Days to Platelet engraftment	16 (11-230)
Complication	
Oral mucositis	23
Hyperbilirubinemia	10
Hypertension	12
Sinusoidal obstruction syndrome (SOS)	1
Infections (number of patients)	26
- Bacterial	11
- Viral	21
- Fungal	3
GVHD	
Acute GVHD	
- Acute Gr I-II	4
- Acute Gr III-IV	0
Chronic GVHD	
- Chronic, limited	3
- Chronic, extensive	0
Alive	36
Cause of death	
Infection	5
Multi-organ failure	3
Severe pulmonary hypertension	1
Follow-up in months	24.9 (7.7 – 67.12)

\*One mosaic patient had late graft failure and one had an early relapse of MDS

Table 1. Multi-institutional study of chemotherapy only preparative regimen for FA: Outcomes

Poster # 500

## DECREASING TIME TO ANTIBIOTIC DELIVERY FOR FEBRILE IMMUNOCOMPROMISED PATIENTS IN A PEDIATRIC EMERGENCY DEPARTMENT

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**Background:** Infections are common complications in immunocompromised patients (ICPs). Morbidity and mortality are increased in ICPs with fever if antibiotics are not received in a timely manner.

**Objectives:** We designed a quality improvement project to reduce antibiotic delivery time in a pediatric emergency department (ED) for this high risk population.

**Design/Method:** Four key drivers were identified to decrease time to antibiotic delivery: patient knowledge, patient identification, antibiotic preparation and patient acuity awareness. To increase patient knowledge, the hematology/oncology service (Heme/Onc) encouraged patients to call with fevers and put EMLA cream on their port site. To increase patient identification, we facilitated direct communication between Heme/Onc and ED attendings. Attending physicians then added the patient to an expected list. "Febrile, immunocompromised" was added to the EMR list of chief complaints for use with ICPs. To address timeliness of antibiotic preparation, a weight-based antibiotic dosing chart was created and displayed at every workstation and in the nursing medication room. The nurses worked simultaneously to triage, obtain blood samples and prepare drugs. To improve awareness of this project, all residents are given a one page summary prior to the start of their ED rotation. Weekly, data driven, informal meetings were held to

provide feedback. As further incentive, nursing staff was provided coffee cards for delivering antibiotics in less than 60 minutes.

**Results:** Mean time to antibiotic delivery in febrile ICPs decreased from 93.32 (SD=63.16) minutes in the pre implementation period to 38.81 (SD=23.48) minutes in the 3 months post implementation ( $p<.01$ ). Effect size associated with the interventions was 'large' (Cohen's  $d=1.14$ ). The percentage of patients meeting the target time rose from 33.3% to 97.9%. After complete implementation of the improvement package, only 1 of 48 febrile ICPs received antibiotics outside the 60 minute goal time.

**Conclusion:** Our study demonstrates that education of healthcare providers and standardization of a process of care reduced antibiotic delivery time for febrile ICPs. Timely delivery of antibiotics can be achieved through implementation of patient education, a treatment algorithm and staff buy in. Administering antibiotics in less than one hour is feasible and should become the standard of care for all febrile ICPs.

Poster # 501

## **ROLE OF PLATELETS IN THE DEVELOPMENT OF GRAFT VERSUS HOST DISEASE**

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**Background:** Thrombocytopenia is a negative prognostic indicator of survival in allogeneic hematopoietic stem cell transplant recipients. When thrombocytopenia is related to chronic graft versus host disease (cGVHD) outcomes are dismal. The correlation between thrombocytopenia is neither clearly explained nor well understood. We sought to investigate the role of platelets in the development of GVHD in a mouse model using platelet factor 4 knockout mice.

**Objectives:** Role of platelets in GVHD

**Design/Method:** We designed an experiment with 4 experimental groups where we manipulated both the marrow source and type of recipient to assess the effect of Platelet factor 4 knockout marrow and recipient animals. We anticipated that all of our experimental groups would develop GVHD. All mice were treated with 700 cGY, and were transplanted with 5 million cells per mouse. Each group contained six mice. The control group was Balb C donor bone marrow cells transferred to Balb C recipient mice. We evaluated four experimental groups. Group 1 was Wild type (WT) C57bl/6 donor bone marrow cells into of Balb C mince. Group 2, Platelet factor -4 Knout (PF-4<sup>-/-</sup>) donor bone marrow into Balb C mice. Group 3 was WT C57bl/6 T cell depleted bone marrow + T cell from spleen into to PF-4<sup>-/-</sup> mice. Group 4, was PF-4<sup>-/-</sup> T cell depleted bone marrow + T cells from spleen injected to WT C57bl/6.

**Results:** All groups were followed daily for survival. Complete blood counts were done at 21 days after the transplant to assess for engraftment and confirmation of thrombocytopenia of the KO group. Disease activity index includes weight loss, loss of fur, hunched back and diarrhea. Disease activity index were monitored twice a week. DAI score 0- 2 Control group has 100% survival without any signs of GVHD. However, unexpectedly pf-4<sup>-/-</sup> groups had less manifestation of GVHD and 70% survived. WT mice had more manifestations of GVHD and 50% survived.

**Conclusion:** Thrombocytopenia considered as a poor prognostic factor in cGVHD, however in this experiment unexpectedly showed that the thrombocytopenic group had fewer manifestations

of GVHD and survived better than the WT group. We plan to investigate this unique observation with further experiments.

Poster # 502

### **FEBRILE NEUTROPENIA AND SICKLE CELL PAIN CRISIS: ACUTE MANAGEMENT PLANNING**

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**Background:** Emergency care in the Pediatric Hematology/Oncology population requires extensive multidisciplinary coordination, education, and communication to maximize patient outcomes. During a major site relocation of our Children's Blood and Cancer Center, a primary safety initiative focused on emergent care for febrile neutropenia (FN) and sickle cell vaso-occlusive crisis (VOC) among our patients. Current accepted national standards for these patients include time to antibiotics in FN <60 minutes and time to opioids in VOC <30 minutes from emergency department (ED) arrival.

**Objectives:** This quality initiative aimed to decrease time to antibiotics in FN and time to analgesia in VOC at or better than national guidelines in 90% of patients within 6 months of site transition.

**Design/Method:** An Emergency Care Taskforce was created several months prior to transitioning. A retrospective review was completed at the prior institution citing current ED time to antibiotics in FN and time to analgesia in VOC. Prospective data was collected similarly after transitioning. Development of fishbone diagram and process flow chart assisted in identifying key players and targeted areas for intervention to improve time outcomes. Implementations included education to all levels of providers (residents, ED providers and nurses) and patient families, a patient identification card for rapid ED triage, evidence-based management algorithms posted in ED work areas and also translated into automated EMR order sets for FN and VOC, and a dedicated Pediatric Hematology/Oncology cell phone to improve communication with hematology/oncology providers.

**Results:** Retrospective review of ED care at the prior institution revealed an average time to antibiotics of 137 minutes in FN and an average time to analgesia of 84 minutes in VOC. However, upon transition to our new site and implementation of the educational and EMR initiatives, ongoing prospective review reveals a significant improvement with time to antibiotics in FN an average 74 minutes and time to analgesia in VOC an average of 67 minutes.

**Conclusion:** Marked reduction was achieved in both time to antibiotics in FN and time to analgesia in VOC. However, current practice does not yet meet the national standard. Next steps include further pre-arrival planning and scheduled ED staff educational sessions with ongoing data review.

Poster # 503

### **FACTORS DETERMINING OUTCOME OF HEMATOPOIETIC CELL TRANSPLANTATION IN PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA AT KING FAISAL SPECIALIST HOSPITAL AND RESEARCH CENTER, RIYADH, SAUDI ARABIA**

Suleimman Al-Sweedan, Amal Al-Seraihi, Asim Belgaumi, Ali Al-Ahmari, Khaldoun Alkayed, Hassan El-Solh, Abudullah Aljefri, Khawar Siddiqui, Rafat Jafri, Mohammad Viqaruddin, Neamah Farhan, Amal Mohammed, Mouhab Ayas, Ashraf Khairy

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**Background:** Hematopoietic cell transplantation (HCT) is used as a viable treatment option for Acute Lymphoblastic Leukemia (ALL) whose disease demonstrates dismal prognosis with chemotherapy.

**Objectives:** Determining factors that affect outcome after HCT.

**Design/Method:** Records of 82 patients with ALL who underwent HCT (2005-2011) were reviewed after approval from institution review board. Data extracted included those related to clinical characteristics of the patients and their outcome.

**Results:** Forty five patients were male (54.80%). Median age at HCT was 7.46 years (range 0.98-14.31), median time to HCT after diagnosis was 12.56 months. Ten patients were below the age of one year (12%). All patients were in complete remission (CR) at the time of HCT. 40 patients were in CR1, 35 in CR2 and 7 in CR3 at the time of HCT. In 83 transplants, 64 patients received HCT from HLA-identical related donors and 19 from other donors (1 or 2 antigens mismatch cord blood [CB] or one antigen mismatch sibling or related bone marrow [BM]). Stem cell source was BM in 65(78%) and CB in 18(22%). All patients were given myeloablative conditioning regimen. Overall (OS) in our patients was 58.8% and event free survival( EFS ) was 54.3%. Median follow-up time for the cohort was 47.2±4.3 months (95%CI: 38.7-55.6) .49 patients were alive with a median follow-up time of 47.2 months (Min: 6.1, Max: 109.9). 17 patients survived for more than 5 years (Min: 61.4, Max: 109.9) months. The cumulative incidence of acute GVHD was 4.8±2.3 and of chronic GVHD was 8.9±3.2. Median time to ANC and platelet recovery was 17 days (range 12-43) and 28 days (range 15-98) respectively. One patient acquired CMV infection after transplant. No one developed VOD, Hemorrhagic cystitis or other complication. Patient's age at HCT and gender, donor's HLA status and gender, source of transplant and CR status at HCT did not significantly affect the probability of OS and EFS.

**Conclusion:** Our results show a favorable outcome to HCT for ALL patients comparable to published data, and no single factor was associated with superior outcome. OS and EFS in cord blood recipient graft is similar to BM recipient graft.

Poster # 504

## **OUTCOMES RESILIENCE AFTER SYSTEM STRESS: A RAPID-CYCLE RESPONSE TO MITIGATE THE IMPACT OF SYSTEM STRESS ON PRIMARY BLOOD STREAM INFECTIONS**

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**Background:** Immunocompromised children are at high risk for primary blood stream infections (BSI) and the associated morbidity and mortality. Prevention of BSIs depends on highly reliable care.

**Objectives:** To rapidly identify the components of a stressed system in order to mitigate the impact of system stress on the rate of BSIs

**Design/Method:** Since the Summer of 2013, we saw a dramatic increase in patient volume and acuity to the Cancer and Blood Disease Institute at Cincinnati Children's Hospital Medical Center. BSI rates more than doubled during this time period. Through a failure-mode analysis, we identified poor adherence to our daily hygiene guidelines, high rates of nurses requiring assistance to complete high BSI-risk procedures, and an unreliable system to escalate concerns from the bedside to unit leadership. Through small tests of change, we implemented a standard process for daily hygiene, increased awareness of high BSI-risk patients, improved education/assistance for nursing performing high BSI-risk procedures, and developed a system to improve allocation of resources to deescalate system stress.

**Results:** Since our mitigation strategies were implemented, we have reduced our primary BSI rate from 1.8 primary BSIs per 1000 line days (7/13-5/14) to 0.18 BSIs per 1000 line days (6/14-12/14) (12000 line days). Key processes have become more reliable: 100% of dressing changes are completed with the new, 2 person standard; daily hygiene adherence has increased from 25% to 70%; 100% of nurses are approached daily by senior nursing daily; and patients at risk for a BSI are identified daily.

**Conclusion:** Since our mitigation strategies were implemented we have decreased our BSI rate 90%. Key processes have become more reliable: 100% of dressing changes are completed with the new, 2 person standard; daily hygiene adherence has increased from 25% to 70%; 100% of nurses are approached daily by senior nursing daily; and patients at risk for a BSI are identified daily. These interventions may have prevented 16 blood stream infections, \$900,000 in healthcare dollars, 350 hospitalization days, and 1-2 patient deaths.

Poster # 505

## **TCR $\alpha\beta$ AND CD19 DEPLETED HAPLOIDENTICAL STEM CELL TRANSPLANT IN HOYERAAL - HREIDARSSON SYNDROME, A USEFUL STRATEGY IN A HIGH RISK GROUP.**

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**Background:** Hoyeraal-Hreidarsson syndrome (HHS) is an extremely rare telomere biology disorder representing the most severe spectrum of dyskeratosis congenita (DKC). Stem cell transplant is the only known cure for marrow failure associated with DKC. Conditioning chemotherapy toxicity is significant hence reduced intensity conditioning (RIC) is recommended. Graft manipulation with TCR $\alpha\beta$  and CD 19 depletion is a novel technique of haploidentical stem cell transplantation, which has recently shown promising results in non-malignant paediatric stem cell transplantation.

**Objectives:** To establish the efficacy of TCR $\alpha\beta$  and CD19 depletion as haploidentical stem cell manipulation technique combined with RIC in an infant with HHS.

**Design/Method:** We describe an infant who presented with prematurity, intrauterine growth retardation and mild neonatal thrombocytopenia that rapidly progressed to severe bone marrow failure. Combination of these features with microcephaly, developmental delay, severe cerebellar hypoplasia and short telomere length confirmed a diagnosis of HHS. Mutation analysis for known genes is negative and result of whole exome sequencing is awaited. We proceeded to urgent haploidentical transplant in view of severe pancytopenia needing frequent blood product infusions and lack of a suitable donor.

**Results:** Haploidentical transplant was performed at 10 months of age using father as a donor

(KIR mismatched and KIR B haplotype). RIC chemotherapy with ATG (Thymoglobulin, 2.5 mg/kg/day X 4 days), Fludarabine (40mg/m<sup>2</sup>/day X 4 days) and Treosulfan (12 gram/m<sup>2</sup>/day X 3days) was used. GCSF mobilized peripheral blood stem cells were collected and processed by negative selection for TCR αβ and CD19. Infused product had CD34+ cells 36 X 10<sup>6</sup>/kg and TCRαβ cells 0.45 X 10<sup>4</sup>/kg. No graft versus host disease (GVHD) prophylaxis was used. Rapid platelet and neutrophil engraftment were noted at D+11 and D+15 respectively. Apart from mild mucositis, one episode of culture negative fever and poor feeding no complications were observed. He is now 10 weeks post-transplant with no infections or GVHD. Day 30 lymphocyte subset analysis shows excellent NK cell reconstitution and 100% donor chimerism.

**Conclusion:** TCRαβ and CD19 depleted Haploidentical stem cell transplantation when combined with RIC offers a useful strategy in children with severe form of DKC who are otherwise considered a high risk group for transplant related toxicity.

Poster # 506

### **PILOT TEST OF A DECISION RULE AND IMPLEMENTATION OF AN OUTPATIENT MANAGEMENT STRATEGY FOR LOW RISK PEDIATRIC FEVER AND NEUTROPENIA**

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**Background:** Evidence-based guidelines support outpatient management of pediatric oncology patients with low risk fever and neutropenia.

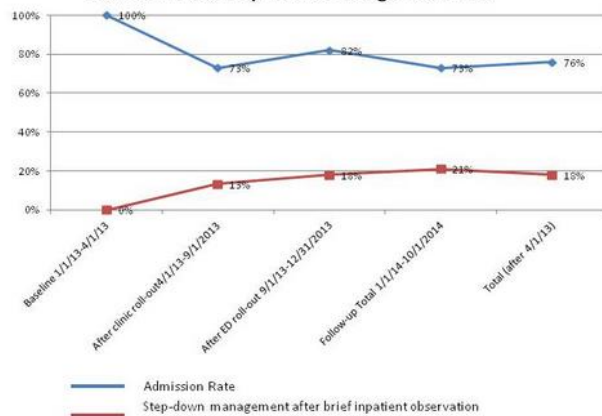
**Objectives:** We aimed to pilot-test a decision rule in our patient population, and then to implement a clinical pathway to reduce our admission rate.

**Design/Method:** We conducted a pilot study of early discharge to home intravenous antibiotics. We then implemented a low risk clinical pathway for outpatient management with oral antibiotics after an initial intravenous dose.

**Results:** Among 35 episodes of fever and neutropenia in our pilot study, there were 2 readmissions and no major medical complications. After implementation of our low risk pathway (**table 1**), our 18-month admission rate declined from 100% to 76% (47 of 62), and another 18% of episodes were managed with a step-down strategy (**figure 1**). One patient required admission for a positive blood culture.

**Conclusion:** Carefully selected patients with fever and neutropenia can be safely managed as outpatients.

**Figure 1. Run Chart of MGHfC Fever and Neutropenia admission and step-down management rates**



**Table 1: MGHfC Protocol for the management of pediatric oncology patients with fever who may be neutropenic and candidates for outpatient management**

**Low-risk criteria for outpatient management:**

- Age > 1 year
- In remission if there is a diagnosis of leukemia (not during induction)
- No acute myeloid leukemia or recent stem cell transplant (in the last 100 days)
- Clinically well-appearing and hemodynamically stable during observation period
- No evidence of serious infection (e.g. cellulitis, concerning abdominal exam, pneumonia on CXR if CXR indicated)
- No other indications for admission (e.g. dehydration, severe mucositis, pain)
- Able to take oral antibiotics
- No contraindication to levofloxacin
- No concern about family's adherence or ability to communicate by phone and return to hospital if necessary

**Treatment Plan:**

Draw stat CBC/diff and blood culture from all lumens of central venous line (CVL)  
 Draw other labs as clinically indicated  
 Give levofloxacin 10 mg/kg IV through CVL  
 Observe for at least 30 minutes after antibiotic completed  
 If eligible for discharge and neutropenic, discharge home on oral levofloxacin until fever and neutropenia have resolved, with daily phone contact  
 Give family the discharge instruction sheet and arrange follow-up

Poster # 507

## A RETROSPECTIVE STUDY ANALYZING THE ADVERSE EFFECTS AND EFFICACY OF IV PENTAMIDINE FOR PNEUMOCYSTIS JIROVECI PNEUMONIA PROPHYLAXIS IN PEDATRIC ALLOGENEIC STEM CELL TRANSPLANT PATIENTS

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**Background:** Pentamidine, administered intravenously or aerosolized, is commonly used for pneumocystis jiroveci pneumonia (PJP) prophylaxis. Very few studies have looked at adverse effects and efficacy of IV pentamidine.

**Objectives:** To determine the rate of breakthrough PJP while on IV pentamidine, to identify risk factors associated with higher rates of PJP breakthrough, and to assess the side effects and tolerability of IV pentamidine in allogeneic hematopoietic stem cell transplant (allo-HSCT) recipients.

**Design/Method:** We retrospectively reviewed the charts of 196 patients who underwent first allo-HSCT between January 2007 and December 2012.

**Results:** Inclusion criteria included allo-HSCT patients (0 to less than 18 years of age) who

received at least 3 doses of IV pentamidine monthly over 3 months between January 2007 and December 2012. We excluded patients with incomplete data of pentamidine administration and/or patients who received IV pentamidine at least 6 or more weeks apart. A total of 144 of 196 patients met criteria. The median age at HSCT was 6 years (age range 0.08-17.87 years). There were 79 males (54.9%) and 65 females (45.1%). The diagnosis prior to HSCT included hematologic malignancies (n=87), bone marrow failure syndromes (n=8), hemoglobinopathies (n=12), immunologic disorders (n=26), and other miscellaneous diseases (n=11). The majority of patients received peripheral blood (57.6%), and the rest received either bone marrow (21.5%) or umbilical cord (20.8%) HSCT. There were no proven or suspected cases of PJP documented. Twenty-nine (20.1%) patients developed a total of 33 side effects to IV pentamidine; respiratory (18.2%), cardiovascular (6.1%), gastrointestinal (15.2%), genitourinary (3.0%), neurologic (27.3%), dermatologic (12.1%), and other (18.2%). No patient discontinued therapy secondary to adverse events.

**Conclusion:** IV pentamidine appears to be well-tolerated and effective for prevention of PJP infection in allo-HSCT. No documented cases of breakthrough infection of PJP were found in our patient population. Thus, we endorse IV pentamidine as PJP prophylaxis for pediatric allogeneic HSCT recipients.

Poster # 508

## BIOMARKERS IN FEBRILE NEUTROPENIA

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**Background:** Febrile neutropenia is a common cause of morbidity and mortality in pediatric oncology. No well-established algorithms using biomarkers exist for risk stratification of these patients. Although not extensively studied in pediatric cancer patients, studies have shown procalcitonin to be a predictor of bacteremia in other settings.

**Objective:** We performed a pilot study to determine baseline values of procalcitonin in pediatric cancer patients and to ascertain the utility of procalcitonin, CRP, and lactate in predicting bacteremia and identifying patients at risk for poor outcomes.

**Method:** We enrolled 43 patients (70.7% leukemia/lymphoma, 29.3% solid tumors, median age 10 years). Procalcitonin was tested in four groups of patients on chemotherapy – afebrile/non-neutropenic, afebrile/neutropenic, febrile/non-neutropenic, and febrile/neutropenic. During each febrile episode, procalcitonin, CRP, and lactate were tested daily for three days.

**Results:** Procalcitonin was not found to vary by gender, age, or diagnosis. Preliminary data suggests that procalcitonin is not elevated in afebrile/non-neutropenic patients ( $p<0.03$ ), although it is elevated in neutropenic patients whether or not they are febrile ( $p<0.03$ ), as does lactate ( $p<0.02$ ). Elevated CRP ( $p<0.05$ ) appears to correlate with presence of fever, while lactate does not. Although not statistically significant, there is a trend toward procalcitonin elevations while febrile ( $p\sim0.055$ ). The negative likelihood ratio of normal procalcitonin values being associated with bacteremia is modest (LR ratio 1.09) as is the negative likelihood value of normal CRP, lactate, and procalcitonin together (LR ratio 1.23), but both are likely affected by the small sample size. Interestingly, in three patients with positive blood cultures, procalcitonin was markedly elevated (in some cases, nearly 70 times the upper limit of normal). However, due to the low prevalence of bacteremia in our participants, no conclusions can be drawn from this observation.



**Conclusion:** Procalcitonin values do not appear to be elevated in “well” oncology patients, making it potentially useful in identifying patients with acute infections. Data suggest that normal procalcitonin may be useful in identifying patients less likely to be bacteremic. Because positive blood cultures are relatively rare events, a larger, multicenter trial is needed to further understand the relevance of extremely elevated procalcitonin levels, elucidate the relevant clinical factors and test them prospectively.

Poster # 509

**INCIDENCE AND OUTCOMES OF PEDIATRIC HEMATOPOIETIC TRANSPLANT PATIENTS DIAGNOSED WITH THE NEW CLASSIFICATION MUCOSAL BARRIER INJURY LABORATORY-CONFIRMED BLOODSTREAM INFECTIONS (MBI-LCBI)**

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**Background:** In 2013, the CDC developed a modification of blood stream infection (BSI) definition, termed mucosal barrier injury laboratory-confirmed bloodstream infection (MBI-LCBI). In 2013, the National Healthcare Safety Network integrated a MBI-LCBI definition into the methods for BSI surveillance to aid in identifying a subset of BSIs reported as central line associated blood stream infections (CLABSI) likely related to mucosal barrier injury. There is little literature describing the timing, patients at risk, and incidence of MBI-LCBIs in comparison to CLABSIs and BSIs stemming from a secondary source (secondary BSIs).

**Objectives:** To determine the timing, underlying diagnosis, and outcomes of patients diagnosed with MBI-LCBIs, CLABSIs and secondary BSIs after stem cell transplant (SCT).

**Design/Method:** We reviewed all BSIs in SCT patients from 5/2011-4/2014 at our institution. We applied the MBI-LCBI classification to patients that met criteria prior to 1/2013.

**Results:** 34 CLABSIs, 30 MBI-LCBIs, and 26 secondary BSIs were diagnosed in 64 patients during the 36-month study time period. Thirteen patients (20%) had more than one infection accounting for 39 of 90 (43%) infections. MBI-LCBIs occurred shortly after SCT at a median of 7 days after transplant. Eleven of 14 patients with MBI-LCBIs after day +30 (including patients with more than 1 infection) were diagnosed with GVHD, with 10/11 having GI-GVHD.

**Conclusion:** One third of BSIs were classified as an MBI-LCBI. MBI-LCBIs occurred earlier after SCT than CLABSIs and secondary BSIs, and were associated with GVHD after day +30. Further research is needed to understand the pathogenesis and prevention of MBI-LCBIs.

**Table 1: Demographics and outcomes of patients with blood stream infections (BSI) after stem cell transplant**

	MBI-LCBI (n=18)	CLABSI (n=20)	Secondary BSI (n=13)	> 1 BSI after HSCT (n=13 patients) *39 BSIs
Median age (years) at SCT (Range)	7.1 (0.9-28.7)	4.6 (0.3-29.8)	5.4 (0.9-32.8)	9.2 (2.2-27.5)
Median days from SCT to BSI (IQR)	7 (4-55)	59 (8-137)	70 (48-118)	149 (58-313)*
<b>Diagnosis</b>				
Immune Deficiency (n=32)	8 (44%)	13 (65%)	4 (31%)	7 (54%)
Malignancy (n=17)	6 (33%)	5 (25%)	3 (23%)	3 (23%)
Marrow Failure (n=8)	2 (11%)	0	3 (23%)	3 (23%)
Benign Hematology (n=5)	1 (6%)	1 (5%)	3 (23%)	0
Genetic / Metabolic (n=2)	1(6%)	1 (5%)	0	0
Myeloablative conditioning (n=28)	8 (44%)	12 (60%)	5 (38%)	3 (23%)
GVHD (Grade II-IV) at 100 days (n=19)	4 (22%)	5 (25%)	6 (46%)	4 (31%)
Sepsis/shock (n=31)	4 (22%)	10 (50%)	3 (23%)	14 (36%)*
ICU care within 7 days of BSI (n=15)	4 (22%)	4 (20%)	3 (23%)	4 (10%)*
Died (n=27)	8 (44%)	8 (40%)	3 (23%)	8 (62%)
Non relapsed mortality (n=20)	6 (33%)	6 (30%)	3 (23%)	5 (38%)
Median days from BSI to death (IQR)	90 (51-152)	119 (50-206)	44 (34-63)	
In patients with > 1 BSI, median time from last BSI to death (IQR)				156 (113-302)

Poster # 510

## THE AMBULATORY CENTRAL LINE ASSOCIATED BLOOD STREAM INFECTIONS BURDEN IN A PEDIATRIC HEMATOLOGY-ONCOLOGY POPULATION

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**Background:** Central line associated blood stream infections (CLABSI) cause morbidity and mortality and increase the costs of care. Inpatient CLABSI (IP-CLABSI) can be reduced by adhering to care bundles. Pediatric hematology/oncology (PHO) patients, including stem cell transplant (SCT) recipients, are a unique population with increased risk factors for infection related to neutropenia and gut compromise. Because PHO patients spend most days outside the hospital with central lines in place for months to years, they are at risk for ambulatory CLABSI (OP-CLABSI). The CDC has reported an average attributable per patient cost of IP-CLABSI ranging from \$7,288-\$29,156<sup>1</sup>. Little work has focused on the impact of OP-CLABSI.

**Objective:** To identify the economic burden associated with OP-CLABSI in a PHO population.

**Design/Method:** We prospectively identified OP-CLABSI events at Dana-Farber Cancer Institute/Boston Children's Hospital (DFCI/BCH) in PHO patients between 1/1/2012 and 12/31/2013. We collected charges for outpatient visits at DFCI, and emergency department (ED) and hospitalizations at BCH related to unique CLABSI events. Visits to outside EDs, cytotoxic medications, MRIs, and nuclear medicine imaging were excluded, since they were likely unrelated to CLABSI events. All room charges were included, although some patients stayed in the hospital for planned treatment in addition to the unplanned CLABSI care. Excel Pivot Tables were used to calculate frequency of visits and charges.

**Results:** There were 71 unique CLABSI events in 59 patients with a mean per patient charge of

\$55,174 (S.D. \$49,955). All patients were admitted to BCH, except one who was receiving palliative care and was managed as an outpatient. The mean length of stay was 9 days (S.D. 8.1). Room (\$1,773,700) and pharmacy (\$892,962) charges accounted for the majority of total charges (\$3,917,344).

**Conclusion:** Preliminary analysis suggests that OP-CLABSI charges exceed IP-CLABSI. This initial approach suggests that OP-CLABSI results in significant additional healthcare cost. Substantial system savings and improved outcomes could be achieved if further efforts are invested in preventing OP-CLABSI. Further detailed analysis is needed to determine the specific costs directly attributable only to OP-CLABSI.

<sup>1</sup>Reference: Scott, CDC, 2009

Poster # 511

## **PROSPECTIVE PILOT STUDY EVALUATING SLEEP DISRUPTION IN CHILDREN AND YOUNG ADULTS UNDERGOING STEM CELL TRANSPLANTATION**

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**Background:** Sleep disruption can adversely affect metabolism, psychological well-being, performance, inflammatory cytokines, immune function, and cardiac function and is likely overlooked as a side effect of stem cell transplantation (SCT). There are no objective studies evaluating sleep in pediatric SCT patients. Actigraphic monitoring utilizes a small monitor that is worn like a wristwatch and when used in combination with self-reports of bedtime and rising time, has been found to be a reliable and valid measure of sleep patterns.

**Objectives:** To evaluate sleep patterns in SCT children and young adults through actigraphy, sleep diaries, and evaluation of environmental factors that influence sleep disruption.

**Design/Method:** Objective sleep parameters were measured with the Micro-Motionlogger SleepWatch® (Ambulatory Monitoring, Inc.) actigraph which was worn continuously. Sleep-wake patterns were reported in a sleep diary to allow for screening of artifacts. The number of waking episodes per cycle of sleep, duration of sleep, minutes of sleep, and sleep efficiency (Abnormal is < 90%) were calculated using actigraphy data. Room entry checklists were utilized to record the frequency of nocturnal room entries in a separate cohort of patients on randomly-selected nights on the BMT unit.

**Results:** We obtained 71 nights of sleep for eight patients. Six patients (75%) were male with a median age of 12.4 years (IQR 10.6-14.1) at time of actigraphy. Thirty-six nights (51%) were pretransplant, 35 nights (49%) were post-transplant. The average sleep onset time was midnight with an average rise time of 9:00 am. The median duration from onset to offset was 9.1 hours (IQR 8.0-10.2) with a median duration of 7.1 hours (IQR 6.1-7.8). The median number of waking episodes per sleep cycle was 12 (IQR 9-17). Median sleep efficacy was 80.7% (IQR 70 to 89%); sleep efficiency was abnormally low 75% of the nights. During 189 nights of room monitoring, patient's rooms were entered a median of 12 times per night (IQR 10-15).

**Conclusion:** These preliminary data suggest that pediatric patients undergoing SCT often have poor sleep quality while hospitalized. Frequent room entries, amongst other causes, may contribute to sleep disruption.

## **A TARGETED QUALITY IMPROVEMENT PROJECT TO INCREASE INFLUENZA VACCINATION RATES IN ACTIVE PEDIATRIC ONCOLOGY AND STEM CELL TRANSPLANT PATIENTS**

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**Background:** Pediatric oncology and stem cell transplant patients (SCT) are at high risk of morbidity and mortality due to influenza, a potentially vaccine-preventable disease. Focused efforts are needed to ensure vaccination during active treatment.

**Objectives:** Vaccinate  $\geq 95\%$  of active patients by the end of influenza season to prevent influenza-related ICU admissions and mortality.

**Design/Method:** Stakeholders were identified and a working group created. Multiple PDSA cycles were used to improve the process across four seasons (2010-11, 2011-12, 2012-13, 2013-14). Definition of target patient population evolved: initially all patients seen within six months prior to and at least once during influenza season (2010-11), patients with  $\geq 2$  appointments over 12 months preceding end of influenza season (2011-12), patients receiving chemotherapy/radiation during and within six months prior to start of influenza season, including those  $\geq 100$  days after SCT (2012-13, 2013-14). A dedicated nurse was assigned to document and track vaccinations. A medical eligibility questionnaire developed for 2010-11 was subsequently adapted to function as a standing clinic order. After 2011-12, additional changes included integration of the vaccination process into routine clinic flow, updating the list of eligible patient's monthly, immunizing inpatients at discharge or during prolonged hospitalizations, measuring and publicizing vaccination rates regularly, sending at least monthly reminder emails to prescribers and nurses identifying non-vaccinated patients, and direct outreach to patients by e-mail and telephone.

**Results:** Vaccination rates were 409/486 (84%) for 2010-11, 675/1064 (63%) for 2011-12, 303/350 (87%) for 2012-13, and 320/362 (88%) for 2013-14. Declination rates were 3% (2010-2011), 3.5% (2011-12), 5% (2012-13), and 3% (2013-14). There was one influenza-related ICU stay and no deaths.

**Conclusion:** Ensuring active oncology and SCT patients receive influenza vaccination requires real-time analysis and interventions, including full integration of the vaccination process into routine workflow, changing culture to make vaccination a priority, and targeting non-vaccinated patients as the season progresses. Including all patients seen in the target population dilutes the ability to ensure vaccination for the highest risk patients. Improvements planned for the 2014-15 season include shortening the analysis and intervention cycles to promote vaccination of all current patients by December 31.

## **THE UTILITY OF BRONCHOALVEOLAR LAVAGE IN HEMATOPOIETIC STEM CELL TRANSPLANT RECIPIENTS**

Adam Duvall, Lili Zhao, John Gonzalez, Daniel Paglia, Gregory Yanik

**Background:** Pulmonary complications are a major cause of morbidity and mortality following hematopoietic stem cell transplantation (HSCT). Broncho-alveolar lavage (BAL) is frequently utilized with a wide variation of diagnostic value.

**Objectives:** To compare a large case series of BAL with concurrent radiographic results and antibiotic pre-exposure.

**Design/Method:** 515 HSCT recipients (median age 50 years, 0-75 years) underwent BAL between 2001 and 2012 at a single center, including 417 following an allogeneic and 98 following autologous transplantation. BAL results were correlated with concurrent radiographic findings; including chest radiographs (CXR) and computed tomography (CT). The median time to BAL was 121 days (0-1854).

**Results:** Infectious pathogens were identified in 27.8% of cases, with *Aspergillus sp.* (n= 37) the most common organism identified. Other common pathogens included *Cytomegalovirus* (n=34) and *Staph aureus* (n=12). Non-pathogenic organisms were identified in an additional 14% of cases. CXR (n=374) performed the week prior to BAL were classified based on the predominant finding, as no significant abnormality 106 (28.3%), diffuse airspace disease (ASD) 183 (48.9%), focal ASD 65 (16.5%), diffuse nodularity 11 (2.8%), or focal nodularity 9 (2.4%). Pathogens were identified in only 26% of CXR's with defined radiographic abnormalities, compared to a 25% rate of pathogen identification in BAL's performed without a concurrent CXR abnormality, p=0.83. There was no correlation between the presence of a fungal, viral or bacterial pathogen and CXR findings. CT scans (n=468) were classified based on presence of diffuse ASD 258 (55.1%), focal ASD 50 (10.7%), diffuse nodularity 145 (31%), focal nodularity 48 (10.3%), bronchiectasis 53 (11.3%), tree-in-bud pattern 37 (7.9%). Radiographic patterns that correlated with identification of bacterial pathogens included diffuse air space disease (p=0.02), and tree-in-bud (p=0.03). No other CT finding correlated with the identification of a viral or fungal pathogen. Prior antimicrobial exposure impacted BAL results, with those BAL performed within 2 days of initiation of broad-spectrum antimicrobials having the highest sensitivity for pathogen identification.

**Conclusion:** The overall incidence of pathogen identification was low with BAL (<30%). There was no correlation of CXR findings with pathogen identification. Only the presence of diffuse air space disease or tree-in-bud formation on CT correlated with bacterial pneumonitis.

Poster # 514

## **PSYCHOLOGICAL DISTRESS AMONG PEDIATRIC CANCER CAREGIVERS**

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**Background:** Pediatric cancer caregivers experience psychological distress both during the course of cancer treatment and after the end of treatment. Yet, few studies of pediatric cancer caregiver distress have been done in the United States.

**Objectives:** Evaluate the association of caregiver socioeconomic (i.e., income) and patient factors (i.e., time since diagnosis) on pediatric cancer caregivers' psychological distress.

**Design/Method:** There were N=366 pediatric cancer caregivers who completed a self-administered questionnaire from July 2010-July 2012. Primary outcome measures were two

subscales from the self-report Impact of Event Scale (IES): “Intrusive” thoughts and feelings (range 0-40) and effortful “Avoidance” of situations that serve as a reminder of the stressful situation (range 0-35), with higher scores indicating greater distress. Multivariable linear regression models were used to calculate coefficients ( $\beta$ ) and 95% Confidence Intervals (95% CI) of IES by socioeconomic and cancer factors.

**Results:** Average caregiver overall IES score was 31.2 (SD=16.9), which is above the cutoff score of 20 designating clinically elevated distress. Mean intrusion score was 18.0 (SD=9.9) and avoidance 12.8 (SD=9.0). Caregivers from households with incomes  $\leq$ \$39,999 reported higher mean scores on the avoidance subscale ( $\beta=3.0$ , 95% CI 0.2-5.8,  $p=0.04$ ) than those with incomes ranging from \$40,000-\$79,000. Caregivers with a child  $<1$  year from diagnosis reported higher mean intrusion ( $\beta=3.0$  95% CI 0.7-5.2,  $p=0.01$ ) and avoidance ( $\beta=2.2$ , 95% CI 0.1-4.3,  $p=0.04$ ) subscales compared to those 1-4 years from diagnosis. Distress was marginally greater among caregivers of acute myeloid leukemia (AML) patients ( $\beta=8.7$ , 95% CI -0.05-7.6,  $p=0.05$ ) compared to acute lymphoblastic leukemia. Caregivers of patients currently ages 0-4 ( $\beta=4.2$ , 95% CI 0.9-7.6,  $p=0.01$ ) and 10-14 ( $\beta=5.3$ , 95% CI 1.8-8.8,  $p<0.01$ ) reported higher intrusion scores than those of patients 15-26 years. Other factors such as insurance, rurality, and religious affiliation were not associated with distress.

**Conclusion:** Caregivers with lower household income, and who care for more recently diagnosed patients, patients with AML, and patients who are younger, experience greater psychological distress. Interventions are needed to ease the psychological stress of pediatric cancer.

Poster # 515

## **OUTCOMES OF ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN PEDIATRIC PATIENTS WITH MYELOYDYSPLASTIC SYNDROMES DUE TO MUTATIONS IN GATA2**

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**Background:** Pediatric myelodysplastic syndromes (pMDS) are clonal hematopoietic stem cell disorders for which hematopoietic stem cell transplantation (HSCT) is the only curative therapy. Germline mutations in GATA2 can lead to pMDS but studies on HSCT outcomes for those patients are limited.

**Objectives:** To describe HSCT outcomes of pMDS patients due to GATA2 mutation.

**Design/Method:** We identified 12 patients with pMDS due to germline mutations in GATA2 that underwent HSCT at our center. All patients were enrolled on the Pediatric MDS and Bone Marrow Failure Registry and underwent rigorous phenotype analysis and standardized pathology review. Data on pre-HSCT therapies, organ dysfunction, conditioning regimen, GVHD prophylaxis, and HSCT outcomes including transplant related toxicity and mortality (TRM) and relapse were obtained.

**Results:** All 12 patients underwent myeloablative HSCT at a median age of 15.9 years with a median follow up of 4 years. There was a male predominance ( $n=8$ ). pMDS diagnoses were refractory cytopenia of childhood ( $n=7$ ) and refractory anemia with excess blasts/AML ( $n=4$ ). One patient had relapsed acute lymphoblastic leukemia. Patient phenotypes included: hydroceles, polyneuropathy, thrombosis, kidney abnormalities, warts, infections and tinea versicolor.

Monosomy 7 was the most common cytogenetic abnormality (n=7). Two patients with pMDS received AML therapy prior to HSCT. Conditioning regimens consisted of cyclophosphamide (CY)/total body irradiation (TBI) (n=7), CY/TBI/ATG (n=2), fludarabine/CY/TBI (n=2) and busulfan/CY (n=1). Sources and donors included bone marrow from matched sibling (n=1) and unrelated donors (n=7) or cord blood (n=4). GVHD prophylaxis consisted of cyclosporine A (CSA)/MTX+/- prednisone (n=9) or CSA/ mycophenolate mofetil (n=3). Seven out of 12 patients are alive. One patient died from relapse and 4 had TRM with respiratory failure, infections and chronic GVHD. Eight patients developed GVHD (acute n=4, chronic n=4). Post-HSCT complications included necrotizing fasciitis, recurrent *Clostridium difficile* infection, leukoencephalopathy, severe gastrointestinal bleeding, squamous cell carcinoma, desmoid tumor, and chronic lung disease requiring lung transplant.

**Conclusion:** HSCT is a curative treatment for pMDS due to GATA2. We observed no relapses in patients transplanted prior to leukemic transformation. Moderate TRM and significant post-HSCT morbidities suggest that reduced intensity conditioning should be considered in future prospective trials for pediatric GATA2 patients with low risk for leukemic transformation.

Poster # 516

### **The Educational Passport for hematology/oncology fellows: A pilot project**

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**Background:** Pediatric hematology/oncology fellows learn through management of patients in the outpatient clinic, inpatient rotations and conference attendance. Faculty observed critical gaps in expected knowledge of fellows. We hypothesized that use of a checklist with core concepts would augment learning.

**Objectives:** To create a learning tool for fellows to guide expected content knowledge, prompt directed reading, facilitate discussion with mentors and encourage consistent teaching for fellows.

**Design/Method:** We developed checklists of communication tasks and knowledge relevant to management of pediatric general oncology (leukemia/lymphoma/solid tumor) patients. Faculty consensus was achieved on checklist items which were collated into a booklet: The Educational Passport (TEP).

Fellow baseline knowledge of TEP topics was evaluated prior to tool distribution via anonymous case-based assessment of common diagnoses. (ALL, Hodgkin lymphoma, neuroblastoma, rhabdomyosarcoma) TEP was then given to all fellows at the start of the academic year. First year fellows had access to the tool during their first clinical year and then completed the case-based assessment at the start of year two of fellowship.

**Results:** Baseline assessment of upper level fellows (n=8) demonstrated excellent understanding of differential diagnosis, baseline testing, therapy toxicity and mechanics of patient-related tasks. Weaknesses included diagnostic evaluation, staging, surgical approach, molecular testing, risk assessment, radiotherapy and late effects of treatment. First year fellows had access to TEP during their clinical year and post-TEP exposure scores and evaluation are ongoing.

Six months after TEP distribution, fellows were surveyed regarding tool acceptance and barriers to use (n=10). The majority (60%) used TEP primarily for personal review. Barriers included forgetting to use TEP during patient care (43%) and lack of document availability when needed (57%).

**Conclusion:** Despite diverse clinical exposure, case-based assessment of fellows revealed significant knowledge gaps. Use of TEP facilitates fellow understanding of education objectives and core concepts. Gaps in case-based assessment promoted directed teaching during conferences. The print format was not utilized as expected, and efforts to develop an electronic format with broader topic coverage are underway.

Poster # 517

## **GENETICALLY SELECTED DONOR CURES FANCONI ANEMIA**

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**Background:** Fanconi anemia is a rare, genetic disorder of DNA repair leading to bone marrow failure. The only permanent cure is hematopoietic stem cell transplantation either with a matched sibling or an unrelated donor.

Pre-implantation Genetic Diagnosis (PGD) can provide a selection of an embryo through in-vitro fertilization process to conceive a future offspring of an affected individual who can serve as a "savior" sibling when no potential donor(s) exist. The incidence of conceiving a "savior" is much higher in cases where maternal age is <37.

**Objectives:** To highlight the successful outcome of a Fanconi anemia patient transplanted with stem cells from the patient's Savior sibling.

**Design/Method:** Eleven year old girl with Fanconi anemia and an abnormal mutation in Fanconi A gene was severely symptomatic with frequent transfusion needs and early iron overload. As a result of no matched siblings and unsuccessful search for a matched unrelated donor, the mother of Fanconi affected patient, who was in her 40s, pursued PGD after thorough counselling. She underwent superovulation process for oocyte retrievals and then fertilization with her husband's sperms via Intracytoplasmic sperm injection (ICSI). The fertilized embryos were developed in the laboratory and then biopsied to rule out Fanconi genetic marker, aneuploidy, and also to detect compatibility as a potential suitable "savior" sibling donor. Serum samples of mother, father and affected patient were collected prior to the process to develop molecular genetic probes for Fanconi.

**Results:** Due to advanced maternal age and fewer available maternal oocytes, the number of non-Fanconi euploid embryos that were compatible for being savior sibling donor were fewer than anticipated. There were only two normal compatible embryos that were transferred back to patient's mother. She successfully conceived and delivered one normal child.

Patient was transplanted with her sibling's combined cord blood and marrow stem cells and remained engrafted with > 95% donor cells and stayed in remission for almost 2 years since transplant.

**Conclusion:** PGD for severe molecular genetic disorders and Pre-Implantation Genetic Screening (PGS) can be utilized to result in excellent and compatible sibling donor. Appropriate counseling and experienced IVF programs and molecular genetic laboratory should be selected.

Poster # 518

## **MEASURING PEDIATRIC HEMATOLOGY-ONCOLOGY FELLOWS' SKILLS IN HUMANISM AND PROFESSIONALISM: A NOVEL ASSESSMENT INSTRUMENT**



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**Background:** Care of children with cancer and blood disorders requires substantial humanism and professionalism, yet educators in pediatric hematology-oncology (PHO) lack rigorously-developed instruments to assess fellows' progress in these domains.

**Objectives:** We developed and piloted a novel self-assessment of humanism and professionalism skills using a national sample of PHO fellows. We performed analyses to measure reliability and validity of our novel instrument.

**Design/Method:** The self-assessment tool (15 items) was administered to 122 fellows from 26 PHO fellowship programs. Fellows rated their own skills in 5 domains: balancing competing demands of fellowship, caring for the dying patient, confronting depression and burnout, responding to challenging relationships with patients, and practicing humanistic medicine. Prior to data analysis, an expert focus group defined threshold scores on the instrument that could be used as a cut-off to identify fellows who need support. The ability of the self-assessment to discern fellows who need support was evaluated by calculating the sensitivity and specificity of the novel instrument, using 3 previously-published scales as gold standards (Jefferson Scale of Physician Empathy, Flourishing Scale, Maslach Burnout Inventory). The project was deemed exempt from IRB review and was conducted in collaboration with the ASPHO Training Committee.

**Results:** Forms were completed by 90 fellows (74% response rate). The self-assessment had high internal consistency reliability (Cronbach  $\alpha=0.81$ ) and was moderately correlated with the Flourishing Scale and Maslach Burnout Inventory (Pearson  $r=0.41$  and  $0.4$  respectively) and weakly correlated with the Jefferson Scale of Physician Empathy (Pearson  $r = 0.15$ ). Twenty-five fellows (28%) scored poorly on one of the three established scales (23 Burnout, 1 Flourishing, and 1 Empathy) and 3 fellows (3%) scored poorly on two or more of the established scales. On the novel self-assessment, 28 fellows (31%) were identified as needing support. The self-assessment had a sensitivity of 50% (95% CI: 31%-69%) and a specificity of 77% (95% CI: 65%-87%) for identifying fellows who scored poorly on at least one of the three established scales.

**Conclusion:** The absence of rigorously-developed assessment instruments limits educators' measurement of PHO fellows' skills in humanism and professionalism. Our novel self-assessment was feasible to administer and demonstrated both reliability and concurrent validity.

Poster # 519

## **SUCCESSFUL BONE MARROW TRANSPLANTATION WITH A REDUCED INTENSITY CONDITIONING REGIMEN FOR HYPEREOSINOPHILIC SYNDROME ASSOCIATED WITH MYELODYSPLASTIC SYNDROME**

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**Background:** Hypereosinophilic syndrome (HES) is heterogeneous myeloproliferative disorder defined by persistent blood eosinophilia, evidence of eosinophil-associated pathology and often end organ damage. This condition, while rare in pediatrics, has been described in association

with myelodysplastic syndrome (MDS) and chromosomal abnormalities, including del(20q). Most patients respond to immunosuppressive therapy, though allogeneic bone marrow transplant (BMT) has been used in select cases of those developing MDS. To date, there have been no reported HES patients under 19 years of age treated with BMT.

**Objectives:** Describe a pediatric patient with HES, associated with MDS that was successfully treated with an allogeneic matched unrelated donor (MUD) BMT using a reduced intensity conditioning (RIC) regimen.

**Design/Method:** Case report

**Results:** A 16 year old female with HES was referred for BMT. Past medical history was remarkable for Stage 3 Wilms tumor diagnosed and treated at three years of age per NWTS-3. At age 9 she developed left-sided weakness; MRI revealed a capsular infarct. A CBC revealed a WBC of 28k with 30% eosinophils. Bone marrow examination demonstrated a clonal population of cells with del-20q12. Genetic testing for PDGFRA was negative. A diagnosis of HES was made and for the following four years she was treated with various immunomodulating agents, including corticosteroids, imatinib, mepolizumab, and ATG, with eventual disappearance of the cytogenetic abnormality. Despite these therapies she developed recurrent strokes and showed progressive ischemia on MRI. At age 15, the abnormal clone (del-20q12) recurred and she was thus referred for MUD BMT. Due to significant pre-transplant co-morbidities, she received a RIC regimen consisting of melphalan, fludarabine, and ATG. Neutrophil and platelet engraftment occurred on d+12 and d+24, respectively. The early post-transplant course was complicated by pulmonary failure necessitating ventilation, and late complications have included mild chronic GvHD. Two years post-transplant she is doing well without recurrence of stroke, eosinophilia or del-20q12s.

**Conclusion:** Clinicians should be wary of malignant transformation in HES. Due to co-existent end organ damage in HES and secondary MDS, fully myeloablative BMT can be excessively toxic. Our case demonstrates the curative potential of a RIC regimen for a pediatric patient with HES.

Poster # 520

## IMPROVING THE CURRICULUM IN THE PEDIATRIC HEMATOLOGY AND ONCOLOGY RESIDENCY PROGRAM

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**Background:** The Pediatric Hematology/Oncology Program at the Hospital for Sick Kids is a Royal College of Physicians and Surgeons of Canada (RCPSC) accredited residency training program that consists of a 3-year, time-based curriculum. This curriculum is built upon a foundation of constructivism with an expectation that residents will learn through experiential learning (Kaufman, 2003). However, a time-based system faces the challenge of incorporating all required content within the curriculum in order to meet accreditation standards and develop competence for subspecialty practice.

**Objectives:** To determine the content needs of learners, based on RCPSC training objectives in order to create a 2-year longitudinal academic half-day curriculum.

**Method:** We administered a needs assessment questionnaire to learners and teachers. We then designed a 2-year longitudinal academic half-day curriculum to incorporate learner and teacher priorities.

**Results:** Twelve subspecialty residents (4 first, 2 second, 5 third and 1 fourth year) and 7 teachers completed the needs assessment. 11/12 (91.7%) residents and 5/7 (71.4%) teachers were aware of the published RCPSC training objectives. 10/12 (83.3%) of residents viewed sessions pertaining to core Hematology/Oncology conditions, chemotherapy and laboratory medicine as important to their learning. 9/12 (75%) residents indicated these medical expert objectives should be reviewed annually. Biannual sessions for specialized topics including radiation therapy and palliative care were deemed acceptable by most residents. Sessions on career development and scholarly activities were less valued among learners. Interestingly, teachers indicated that these topics were not adequately covered in the existing program. 11/12 (92%) residents found case-based learning useful whereas only 7/12 (58%) found didactic lectures to be useful. In contrast, 6/7 (86%) of teachers found didactic lectures as a useful teaching method. The academic half-day curriculum map developed based on collaboration with learners and teachers reflected the learning content and teaching methods residents valued.

**Conclusion:** The challenge of curriculum design within a time-based structure will always include the task of covering the enormous amount of content within a curriculum to fulfill both learner and teacher goals. Moving forward, we propose the consideration of a competency-based framework that can be built within a time-referenced program to enhance achievement of minimal competency in all necessary content areas.

(Kaufman,BMJ, 2003)

Poster # 521

## **SINGLE INSTITUTION EXPERIENCE USING ALLOGENEIC HSCT TO TREAT PEDIATRIC NK/T CELL LYMPHOMA**

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**Background:** Extranodal NK/T cell lymphoma, nasal type (ENKTCL-NT), is an aggressive malignancy that typically presents in the nasal sinus regions but can also involve skin, lungs, gastrointestinal tract and other organs. It is most frequently diagnosed in East Asia and South America and is almost always associated with EBV infection. It is rare in Caucasian patients and in developed countries. Treatment is not standardized and mortality rates are high.

**Objectives:** We present a series of three patients diagnosed with ENKTCL-NT at our institution during a ten month period. Each patient was treated with chemotherapy induction followed by allogeneic HSCT consolidation.

**Design/Method:** Patients 1 and 2 were both of Native American descent and patient 3 is Caucasian. Patient 1 presented with a primary nasal mass and metastatic disease to regional nodes and soft tissue of the right forearm. Patient 2 presented with small bowel obstruction and a left gluteal mass. Patient 3 presented with respiratory failure due to diffuse malignant infiltration of the lung parenchyma and secondary hemophagocytic lymphohistiocytosis (HLH). All patients had biopsy-proven ENKTCL-NT. Each patient was treated with SMILE (Dexamethasone, Methotrexate, Ifosfamide, L-asparaginase, Etoposide) chemotherapy and went on to allogeneic bone marrow transplant in CR2, PR1 and PR1 respectively. Following transplant, treatment with donor-derived, EBV-specific, cytotoxic T-lymphocytes (CTLs) was planned for each patient.

**Results:** Both patients 1 and 2 relapsed following bone marrow transplant and died of progressive lymphoma, patient 1 at 6 months post-transplant despite CTLs, and patient 2 on day +30 prior to CTL therapy. Patient 3 was found to have biopsy-proven splenic relapse lesions on day +57 and is currently awaiting CTL therapy.

**Conclusion:** EBV-associated ENKTCL-NT is a rare and aggressive malignancy. We discuss our institution's experience treating the disease and the potential role HSCT may play as a platform for cellular immunotherapy. Although treatment was unsuccessful, our series suggests that more aggressive induction therapy and earlier institution of EBV-specific immunotherapy may be future avenues to pursue.

Poster # 522

## **ANNUAL BLOOD PRODUCTS CONSENT FOR INPATIENT HEMATOLOGY, ONCOLOGY, AND BONE MARROW TRANSPLANT PATIENT: ADVANCED QUALITY IMPROVEMENT PROJECT**

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**Background:** Texas Children's Hospital requires new blood products transfusion consent be obtained upon each inpatient admission. This inefficiency in clinical care management may delay care (blood product administration), increase family "consent fatigue," increase nursing/provider miscommunications, and add frustrations given limited time and resources.

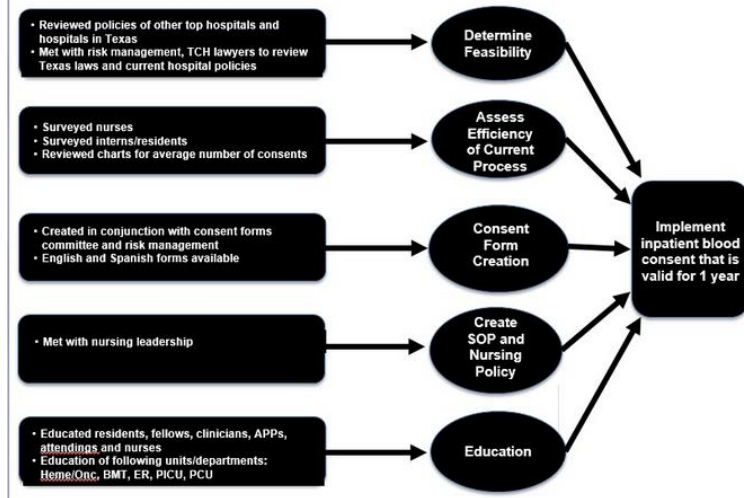
**Objectives:** Change the blood products consent process for hematology, oncology, and bone marrow transplant inpatients to allow for annual consents. This will: 1) Reduce the number of overall treatment consents; 2) Increase provider availability, while decreasing provider non-billable time; 3) Improve provider and nurse communication; and 4) Reduce delays in blood product administration.

**Design/Method:** For PDSA Cycle #1: 1) Consent practices at other children's hospitals were assessed; 2) Pre-implementation surveys were obtained from providers and nurses regarding satisfaction, causes for blood transfusion delays, and frequency of nurse/provider contact regarding consents; 3) Number of blood consents and length of time spent obtaining consents (N = 40 patients) over 6 month period was assessed. The new annual blood products consent form was developed and implemented.

**Results:** Prior to the annual consent form, 40 patients were consented for blood product administration 139 times over 6 months, with total provider non-billable time of 33.7 hours. Blood product administration was delayed an average of 30 minutes per patient due to lack of consent documentation. Post-implementation results/PDSA Cycle #2 are still pending.

**Conclusion:** Creation of an annual inpatient blood products consent form decreases provider non-billable time, decreases patient/family consents, improves nursing and provider satisfaction, and minimizes delays in blood product administration.

## Plan: Drivers Diagram



Poster # 523

## NOVEL APPROACH TO A RARE DISORDER OF GLYCOLYSIS "TRIOSEPHOSPHATE ISOMERASE I (TPI1) DEFICIENCY" - FIRST REPORTED BONE MARROW TRANSPLANT (BMT)

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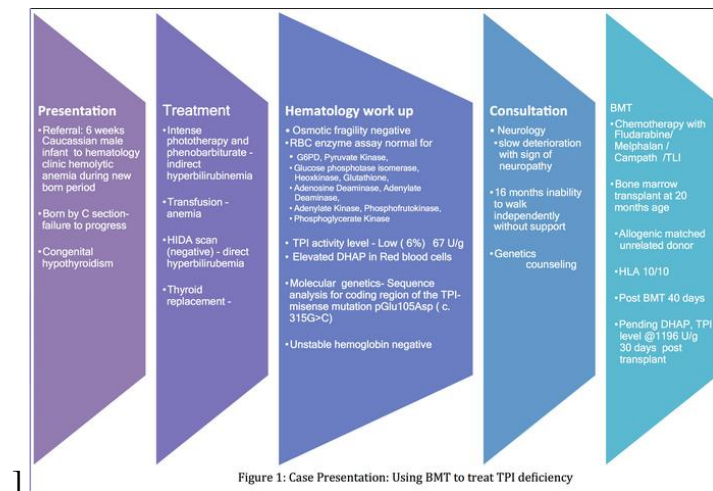
**Background:** Evaluation of congenital hemolytic anemia may include assays for certain enzyme levels. Triosephosphate Isomerase (TPI) deficiency is a rare, autosomal recessive, inherited glycolytic pathway enzymopathy. It presents with jaundice, susceptibility to infection, cardiomyopathy and neuromuscular dysfunction. Only ~50 cases of TPI deficiency are reported in the literature. TPI interconverts dihydroxyacetone phosphate(DHAP) and D-glyceraldehyde 3-phosphate in the glycolytic pathway. Neuromuscular failure leads to respiratory failure and death, often by 6 years. Treatment is supportive and includes splenectomy and red cell transfusions to improve anemia. In-vitro studies indicate plasma borne enzyme may be taken up by deficient tissue. Suggesting that hematopoietic cell derived enzyme could reverse the metabolic deficiency.

**Objective:** To describe a case of TPI deficiency that was successfully treated with Bone Marrow Transplant (BMT)

**Design/Method:** Case-report, Fig.1 shows the detail of the case. The patient was diagnosed soon after birth with hemolytic anemia. Workup revealed low level of TPI activity and elevated DHAP level in RBCs (>100 fold). Homozygous pGlu105Asp mutation was detected. Exam was notable for gross motor delay and evidence of neuropathy. MRI showed delayed myelination. In an attempt to halt progression of disease, we conditioned the patient for BMT. He received a 10/10 HLA matched unrelated bone marrow stem cell product at 20 months of age.

**Results:** At this time, patient is approximately 40 days from transplant. Evaluation is ongoing for clinical improvement and DHAP levels.

**Conclusion:** This case represents one with homozygous TPI deficiency treated with reduced intensity, matched unrelated donor BMT.



Poster # 524

## USING A WIZARD OF OZ APPROACH TO STUDY THE EFFECTS OF EHRs ON CLINICAL WORKFLOW IN A PEDIATRIC ONCOLOGY CLINIC

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**Background:** The implementation of electronic health records (EHRs) promise improvements in the quality and safety of healthcare by providing easy access to information, providing clinical decision support at the point of care, and by allowing the automated tracking of outcomes and care. However, these systems are often felt to be intrusive and time consuming to users. Understanding user cognition in complex systems is central to ensuring system United States ability and implementation success.

**Objectives:** We used methods from cognitive science research to study healthcare provider interaction with the EHR in order to identify areas in which clinical efficiency can be improved.

**Design/Method:** We performed structured interviews with 16 hematology/oncology providers in an ambulatory clinic. Each subject was given 2 patient scenarios representing common visit types in the outpatient pediatric hematology/oncology clinic. During one scenario, we used a “wizard of Oz” approach to learn the user’s ideal workflow in a “perfect system.” In the other, we performed cognitive walkthrough in the clinic’s EHR training environment. The interviews were video recorded and transcribed.

**Results:** In the scenarios performed in the current EHR’s training environment, the order of task completion was determined largely by the build of the system. In the ideal scenarios, providers did not consistently follow this order. For example, they were more likely to review the patient’s historical information prior to reviewing the current visit data. In addition, the ideal systems described by the providers elucidated four areas of need: better visualization tools providing comprehensive summaries of patient histories and data, improved data entry at the point of care, efficient communication tools between care providers, and the addition of patient involvement in the EHR system. Subject matter experts reviewed the workflows to identify deviations in task

completion.

**Conclusion:** Cognitive task analysis is an effective way of identifying gaps in the build of an EHR. It was useful in understanding ways in which the EHR causes deviations from providers' cognitive models of a patient encounter. In addition, we identified further areas of need in the EHR.

Poster # 525

## **EVANS SYNDROME FOLLOWING MATCHED SIBLING ALLOGENEIC STEM CELL TRANSPLANT IN PEDIATRIC PATIENT**

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**Background:** Autoimmune Cytopenia (AC) following matched related donor hematopoietic stem cell transplantation (HSCT) has been reported in adult literature but has not been reported in the pediatric literature. Adult literature suggests post transplant occurrence of Evans syndrome (ES) to be approximately between 3 to 5% and has poor outcome (1,2,3). The incidence of Autoimmune Hemolytic Anemia (AIHA) reported in pediatric patients who received unrelated cord transplant for metabolic disease (4) was four times higher than patient who received transplant for malignant disorder.

**Objectives:** To report a rare case of Evans Syndrome following Matched Sibling Allogeneic stem cell transplant (MST).

**Design/Method:** Case report: We report a rare case of ES with later onset of Neutropenia, following MST for Acute Myeloid Leukemia in a 7 year old girl. Patient received 10/10 human leukocyte antigen & blood type-matched BMT. She was engrafted for ANC on day 17 and platelets on day 28. She also developed grade-2 graft versus host disease. However later, post BMT day 47 she developed Evan's syndrome. Direct Coombs and antibodies directed at platelet glycoproteins (IIb/IIIa, Ia/IIa, Ib/IX) test were positive. Bone marrow was negative for leukemia. Patient responded well to a rapid withdrawal of calcineurin inhibitor (CNI), tacrolimus and treatment with IVIG and a course of steroids.

**Results:** Patient has had complete recovery. Patient remains in full remission with no evidence of cytohemolysis or leukemia.

**Conclusion:** Case reports of ES following MST, especially in the pediatric population are extremely rare. The fact that AC onset in this case occurred after MST suggests that the pathogenic mechanism may differ from that of previously reported cases of ES following unrelated HSCT. Pathogenesis of AIHA ES after HSCT in the present case was unclear and could be from Immune dysregulation brought about by excessive immunosuppression during BMT.

**Reference:** Dovat et al, Bone Marrow Transplant, 1999

1. Mullen et al, Bone Marrow Transplant, 2000
2. Hongeng et al, Bone Marrow Transplant, 2002
3. T.A O'Brein et al, British journal of hematology, 2004

Poster # 526

## NEEDS ASSESSMENT STUDY FOR COMPLIANCE RATE IN PATIENTS WITH MALIGNANCIES AT A SEMI-URBAN SETTING OF INDIA

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**Background:** Developing countries bear a disproportionate burden of cancer. In India alone 700,000 new cancer cases and close to 350,000 cancers related deaths are reported each year. Curable cancers become fatal in developing countries because of low patient compliance and increased fall out rate.

Besides treatment compliance, medication compliance is also problematic in these areas. Multiple studies report noncompliance rates as low as 30% and as high as 50% or above in these areas. Reasons for poor compliance rates after diagnosis and induction therapy in patients with cancers eludes the physicians in resource limited countries.

**Objectives:** Primary - To elucidate the perception of barriers to care in this region.

Secondary- Engaging stakeholder participation in designing interventions to improve treatment compliance.

**Design/Method:** An epidemiological tool combining open ended and Likert scale style questions was given as a self-administered survey to 41 patients with cancer undergoing inpatient treatment at the only tertiary care cancer center in the city of Jaipur. Demographics, expected length of stay, expected cost of treatment, expected length of treatment, perceived and actual barriers to access to cancer care in this setting were assessed. The data was blinded and randomized to the target participants.

**Results:** While 82% patients reported not knowing the exact length of their treatment, 85% reported complete understanding of their diagnosis. Not surprisingly 19% reported knowing the entire duration of treatment to the actual median of 2 years. 5% (4/40) of responders refused to return for follow up care due to access and cost issues. Close to 95% of participants agreed to intended benefit from availability of a mobile health vehicle and free transportation services. 10% of responders suggested integration of cancer care within the institution for better compliance.

**Conclusion:** Incomplete understanding of the treatment, fragmented care within the institution and transportation access were revealed as the major barriers in this pilot study. Much of the developing world share common challenges and with proposed interventions such as mobile health vehicles training mid-level health providers and adequate discharge planning can not only improve compliance but also reduce relapse rates and cancer related mortality.

Poster # 527

## A PROSPECTIVE ANALYSIS OF IMMUNE RECONSTITUTION IN PEDIATRIC PATIENTS UNDERGOING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION

Jennifer Whangbo, Haesook Kim, Katharine Dusenbury, Carol Reynolds, Marie Chammas, William Haining, Suneet Agarwal, Inga Hofmann, Sung-Yun Pai, Michelle Lee, Steven Margossian, Christine Duncan, Leslie Lehmann, Jerome Ritz

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**Background:** Delayed or impaired immune reconstitution (IR) following allogeneic hematopoietic stem cell transplantation (alloHSCT) is associated with the development of graft-versus-host disease (GVHD), infections and relapse. Most IR studies have been performed in adult patients.

**Objectives:** (1) Perform a prospective analysis of IR in pediatric alloHSCT patients. (2) Examine IR following alloHSCT for malignant versus non-malignant hematologic diseases. (3) Compare the kinetics of IR in adult and pediatric alloHSCT patients. (4) Correlate IR with clinical outcomes including GVHD, relapse and overall survival.

**Design/Method:** Patients undergoing alloHSCT at Boston Children's Hospital were offered enrollment on a prospective tissue banking protocol that obtains blood samples at 0, 1, 2, 3, 6, 9, 12, 18, and 24 months post-transplant. IR at each time point was evaluated by multiparameter flow cytometry to enumerate phenotypic and functional subsets within T, B, NK and dendritic cell compartments. Additional markers quantified recent thymic emigrants (RTE), proliferation and susceptibility to cell death within each T cell subset.

**Results:** To date, 34 patients (median age 13 years, range 1-23) have enrolled with a median follow up time among survivors of 8 months. Time for the median cell count to reach the normal range was 9 months for CD4+ and CD8+ T cells, 6 months for CD19+ B cells and 1 month for CD3- CD56+ NK cells. No significant differences in IR were detected in the T cell compartment of pediatric patients with malignant versus non-malignant disease at 6 months, but the comparison is limited by the small sample size of the non-malignant cohort (n=7). Surprisingly, recovery of CD4+ regulatory T cells (Treg) was relatively rapid (6 months for the median cell count to reach the normal range). There was no difference in RTE recovery within the Tcon and Treg compartments in pediatric patients, in contrast to adults, where recovery of RTE within the Treg compartment is often delayed.

**Conclusion:** Comprehensive analysis of IR in pediatric patients undergoing alloHSCT can elucidate the contributions of a functional thymus and provide insights into how this impacts recovery of different T cell subsets. Analysis of clinical correlations with IR is ongoing and requires longer follow up.

Poster # 528

## **PEDIATRIC ONCOLOGY CLINICAL CARE: A NOVEL MODEL TO ACHIEVE BETTER CONTINUITY OF CARE IN A MEDIUM SIZED PROGRAM**

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**Background:** Outpatient pediatric oncology programs currently operate by one of two physician models of care - a primary-physician model employed by large programs, and a team-based model employed by small programs. Medium sized programs (50-100 newly diagnosed patients/year), face a challenge as to the best model of care to follow.

**Objectives:** To develop a hybrid team-based and primary-physician model to improve the continuity of care in a medium sized center.

**Design/Method:** Prior to making any changes from a team-based model of care, a patient/family satisfaction survey was conducted. Using a continuous improvement approach, the team decided that all patients would have a primary oncologist to increase continuity of care. However, the consensus was that weekly clinics would not be allow for academic pursuits, thus the group

developed 2 types of weekly clinical schedules – each offered on alternating weeks. During the first week, each primary oncologist would be in clinic on a consistent day and would see their assigned patients –labeled a “Doc of the Day” week . During the second week, one oncologist would preside in clinic for the entire week and would follow the care plans outlined by the patients' primary oncologists – called a “Doc of the Week” week. Fourteen months after the launch of the new model-of-care, the patient/family satisfaction survey was repeated.

**Results:** Fourteen months after changing the model-of-care, the patient satisfaction survey indicated continued high level satisfaction with perceived oncology care. In addition, at the same time a questionnaire was administered to the multidisciplinary team, which revealed a perceived increase in continuity of care, increased efficiency in clinic work flow and increased consistency of care. Furthermore, having a primary oncologist assigned to each patient facilitated the planning and delivery of patient care and ensured more consistent answers to patient care questions.

**Conclusion:** We demonstrated that a hybrid physician model-of-care that combines: 1)a primary oncologist for each patient; 2)assigned clinic days for each oncologist; and 3)alternating weeks where one physician covers the outpatient clinic for all oncology patients is a feasible, flexible, and increases the continuity of care for patients in a medium sized pediatric oncology program.

Poster # 529

## **AN ACCURATE AND RAPID COLOR-BASED VISUAL POINT-OF-CARE ASSAY FOR THE DETECTION OF SEVERE ANEMIA IN LOW RESOURCE SETTING**

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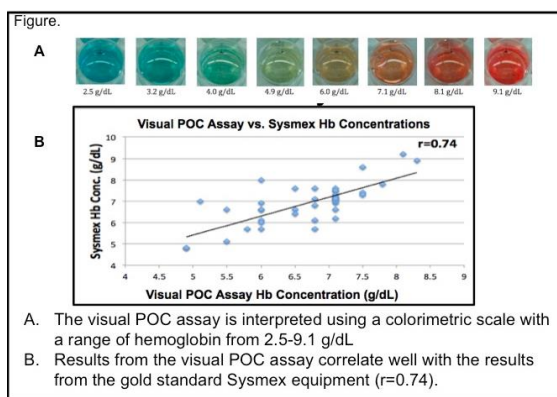
**Background:** Severe anemia is a leading cause of morbidity and mortality among children in low resource settings, but laboratory diagnostics are often quite limited in these locations. A simple, rapid, accurate, and disposable visual point-of-care (POC) anemia assay has recently been developed. This visual POC assay is inexpensive (estimated cost < \$0.25), self-contained in a plastic tube and does not require electricity or complicated sensors and was designed to detect clinically relevant severe anemia (Hb 2.5-9.1 g/dL) in low resource settings.

**Objectives:** To evaluate whether a visual POC assay is able to detect severe anemia as accurately as currently used laboratory techniques in a low resource setting.

**Design/Method:** The study was performed in the sickle cell clinic at Hospital Pediátrico David Bernardino in Luanda, Angola. After receiving informed consent, blood was collected and hemoglobin was determined by: 1) visual POC assay; 2) BioSystems Hemoglobin Analyzer (current standard of care); 3) Sysmex hematology analyzer (more expensive equipment, considered gold standard for comparison).

**Results:** Samples were collected from 40 children. The hemoglobin values (range 4.8-9.2 g/dL) obtained from the visual POC assay correlated well with the Sysmex results ( $r=0.74$ ) and were more accurate than the current standard of care ( $r=0.47$ ). The visual POC assay was accurate across the range of hemoglobins with a mean difference of 0.47 g/dL compared to the Sysmex results.

**Conclusions:** These pilot data demonstrate that a novel visual POC assay is able to accurately detect severe anemia and has the potential to become a useful diagnostic tool in limited resource settings.



Poster # 530

## BREAKING TRADITION: COMPLEMENTARY ROLE OF TRADITIONAL MEDICINE IN NATIONAL CANCER CONTROL PLANS GLOBALLY

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**Background:** Traditional and complementary medicine (T&CM) constitutes an increasingly sought after and recognized aspect of health services globally. The World Health Organization (WHO) has advocated for an integrated approach to T&CM for patients with non communicable diseases (NCD) including cancer.

**Objectives:** We analyzed inclusion of T&CM in national cancer control plans to identify vital gaps and opportunities for improved integration.

**Design/Method:** All national cancer control and NCD plans describing cancer which were publically accessible via the International Cancer Control Partnership Portal were screened. Inclusion of T&CM in each plan was evaluated based on the WHO Traditional Medicine Strategy 2014-2023 strategic objectives and critical indicators.

**Results:** Seventy national plans were accessible for review, from Africa (n=18), Americas (n=11), Asia (n=10), Europe (n=21), and Oceania (n=10); representing World Bank high-income (n=26, 37%), upper-middle-income (n=23, 33%), lower-middle-income (n=9, 13%), and low-income economies (n=12, 17%). Two regional plans were additionally included (continental Africa and regional Pacific islands), totaling 72 plans. 48 plans focused on cancer only, and 24 on NCD; plans were reviewed in English (74%), French (17%), Spanish (10%), and Portuguese (1%). In 43 plans (60%), none of the seven WHO critical indicators across three strategic objectives were addressed. Integrating T&CM into national health services was described in 25 plans (35%), followed by consumer education initiatives in 14 (19%); both reflecting WHO strategic objective 3; several plans specified research funding for T&CM (n=9, 13%) and T&CM policy (n=9, 13%) reflecting strategic objective 1. A few plans described regulation for T&CM products and practice (n=6, 8% for both) and practitioners (n=3, 4%), reflecting WHO objective 2. The context for emphasizing T&CM within 33 plans included three themes: 1) acknowledgment of existing T&CM practices and influences on health behaviours in local contexts; 2) the potential role of appropriately integrated T&CM in decreasing morbidity and mortality, impacting cancer and NCD patients' care trajectory from prevention to treatment and end-of-life care; and 3) the importance of engaging patients in informed therapeutic decision-

making encompassing T&CM.

**Conclusion:** Opportunities exist to promote T&CM in cancer control planning and implementation across diverse settings globally, including improvements in policy, regulation, and service integration.

Poster # 531

## **EFFICACY AND SAFETY OF INTRAVENOUS FERRIC CARBOXYMALTOSE IN CHILDREN WITH IRON DEFICIENCY ANEMIA UNRESPONSIVE TO ORAL IRON THERAPY**

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**Background:** Initial therapy for iron deficiency anemia (IDA) is oral iron. Yet, many patients fail to respond due to poor adherence and/or adverse effects. Ferric carboxymaltose (FCM, Injectafer<sup>®</sup>) is a new (FDA approved in 2013) intravenous (IV) iron preparation with demonstrated safety and efficacy in adults with IDA. Limited data exist on its use in children.

**Objectives:** To assess the hematologic response and adverse effects of IV FCM in a diverse population of children with IDA who failed oral iron therapy.

**Design/Method:** All children with IDA who received FCM at Children's Health Dallas from June 1 to December 31, 2014 were included. Subjects were identified via search of pharmacy records. All patients received at least one dose of FCM 15 mg/kg (maximum 750 mg) administered by a 15-minute IV infusion (without test dose or pre-medications).

**Results:** Sixty-seven infusions of FCM were administered to 47 patients (age 20 months to 20 years, median 13.8 years) during the study period. Etiologies of IDA included nutritional (N = 14), heavy menstrual bleeding (N = 18), GI bleeding or malabsorption (N = 6) and other/mixed cause(s) (N = 9). The median dose administered at a single infusion was 700 mg (range 150 to 750 mg). No adverse effects were noted during or following the infusion in 42 subjects. Two patients had transient tingling, nausea and/or mild abdominal pain. Three adolescents had more clinically significant reactions, 1 with nausea/vomiting post-infusion requiring admission, 1 with urticaria 20 minutes post-infusion, and 1 with dyspnea 2 minutes into the infusion, requiring its immediate termination. The latter two received diphenhydramine, methylprednisolone and saline with prompt symptom resolution. Median pre-infusion hemoglobin concentration was 9.2 g/dL (range 3.9 to 13.3 g/dL). A post-infusion hemoglobin concentration was available for 29 patients from 1 to 16 weeks post-initial infusion with median increase of 3.1 g/dL (range -0.2 to 7.4 g/dL).

**Conclusion:** Intravenous FCM, administered in the outpatient setting as a short IV "total dose" infusion and without need for a prior test dose, is safe and effective in the large majority of children and adolescents with IDA refractory to oral iron therapy.

Poster # 532

## **DEVELOPING A MOBILE HEALTH APPLICATION: INHERENT TECHNOLOGICAL, PROGRAMMATIC, AND DEVELOPMENT ISSUES FROM THE PROVIDER'S PERSPECTIVE**

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**Background:** Mobile technology is increasingly utilized in healthcare to improve treatment delivery and increase patient-provider communication. For sustainable integration, mobile apps require appropriate facility support and patient-provider utility. Current research cites security, liability, sustained use, and patient/provider engagement as barriers to implementation; however, few provider-developed mobile applications have been described.

**Objectives:** We aim to describe potential issues with the development of a mobile health application by medical professionals, including programmatic, development, and implementation hurdles specific to the development of an app for clinical use.

**Design/Method:** Formal and informal descriptive data were collected for qualitative analyses during a pilot study (n=10) utilizing our self-developed application. Post-intervention feasibility measures were collected, providing quantitative data regarding technological issues.

**Results:** *Technological.* Patients utilized a variety of devices and the application required compatibility with several operating systems (iOS5 – iOS8) as well as reinstallation following the release of iOS updates.

Programming an application for online and offline use is challenging due to data storage, data transfer and data backup strategies. Solutions included providing institutional devices and data limitations (such as a maximal video length).

Specific encryption programming for static data storage and data transmission is necessary to ensure adequate security. Despite well-supported encryption platforms in iOS7 and iOS8, most programming development tools do not standardize encryption practices. Programming for privacy and security had to be balanced with usability.

We provided devices to patients with non-iOS devices (approximately 20%). We focused initially on a well-developed single OS app, with plans for future extension.

During 30-day United States, patients endorsed the following via feasibility questionnaire: problems with logging in (10%), uploading data (20%), receiving provider-directed messages (50%), and the app not working every time (25%). Despite these issues, patients' caregivers (n=8) reported a high level of utility (87.5%) and low level of disruption to implement the app (12.5%). Such direct feedback from patients enabled real-time programming adjustments.

**Conclusion:** We successfully developed a mobile app by addressing the above described issues. We hope to provide instructional utility for providers aiming to develop, program, and integrate mobile health technology successfully into clinical practices.

Duke University Medical Center, Durham, NC

Poster # 533

## **CLINICAL PRESENTATION AND OUTCOME OF AUTOIMMUNE HEMOLYTIC ANEMIA IN THE PEDIATRIC POPULATION; EXPERIENCE AT MAYO CLINIC**

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**Background:** Autoimmune hemolytic anemia (AIHA) in the pediatric population is rare with an annual incidence of 0.2 cases per million individuals <20 years.<sup>1</sup> There are limited published data.

**Objectives:** To describe the clinical presentation and outcome of AIHA among the pediatric

population seen at Mayo Clinic, Rochester, Minnesota.

**Design/Method:** We performed a retrospective study of patients age  $\leq 18$  years with AIHA who were seen from 1994-2014. We defined AIHA as the presence of all the following: anemia (hemoglobin  $< 12$  g/dL), positive direct antiglobulin test, and hemolysis. We classified treatment response as complete (CR; hemoglobin  $\geq 12$  g/dL) or partial (PR; 10-11.9 g/dL).

**Results:** We included 35 patients in the study. The median age at diagnosis was 10 years (range, 0.5-18) and 66% were males. The distribution by subtype was warm (78%), cold agglutinin disease (11%), mixed (4%), and paroxysmal cold hemoglobinuria (7%). Ten patients (29%) had secondary causes including viral infections (5), systemic lupus erythematosus (3), rheumatoid arthritis (1) and drug-induced (1). In seven patients, there was concomitant immune thrombocytopenia (Evans syndrome). The median hemoglobin at diagnosis was 6.1 g/dL (range, 3-11.1) and 22 patients (63%) required red cell transfusions. Hemoglobin at diagnosis was lower in children  $< 10$  years (median 5.5 versus 7 g/dL;  $P = 0.01$ ). Steroid was the initial treatment for 31 patients (89%) producing an overall response rate of 81% (68% CR; 13% PR) with a median response duration of 8 months (range, 0.2 to 129.7+). For the remaining 4 patients, the initial treatments were intravenous immunoglobulin (IVIG; 1 CR) or rituximab (1 CR; 1 PR; 1 no response)  $\pm$  steroids. After a median follow-up of 26.6 months (range, 0.5+ to 130.7+), 8 patients (23%) relapsed. Salvage treatments included splenectomy, IVIG, rituximab, and mycophenolate mofetil. Infectious complications occurred in 9 patients post immunosuppressive therapy. One patient (diagnosed at age of 10 months) died of treatment-related cytomegalovirus infection.

**Conclusion:** AIHA is rare in our pediatric population. It is mostly idiopathic in nature and unlikely an initial presentation of a hematologic malignancy. Children under 10 years of age may have a worse presentation. Steroid was the most commonly used treatment.

**Reference:** 1. Aladjidi, Haematologica, 2011

Poster # 534

## COMPARISON OF CLINICAL RESPIRATORY SCORE AND PATIENT OUTCOMES WITH SIMPLE VERSUS RED CELL EXCHANGE TRANSFUSION IN ACUTE CHEST SYNDROME

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**Background:** Acute Chest Syndrome (ACS) is a major cause of morbidity and mortality in patients with sickle cell disease. ACS is the most common cause of death and the second most common cause of hospitalization among the pediatric population. Few studies compare patient outcomes following simple transfusion (ST) versus red cell exchange (RCE).

**Objectives:** The purpose of this study is to compare outcomes and determine if certain measurements predict which patients would benefit from RCE.

**Design/Method:** We conducted a retrospective chart review of confirmed ACS cases at Miami Children's Hospital (Miami, FL) from 1/1/00 through 11/30/14. Differentiating patients who received ST versus RCE, we identified objective factors used in evaluating severity and outcome in ACS. We also applied the Clinical Respiratory Score (CRS) to the dataset retrospectively using a published scoring rubric [1], which includes respiratory rate and effort, auscultation, color change, mental status, and pulse oximetry. Additionally, the comparisons of means were performed by fitting linear regression models.

**Results:** Patients who received an RCE (N=9) had a higher CRS (5.11 vs 3.11,  $p<0.01$ ) and significantly longer hospital stay (11.1 vs 7.5 days,  $p<0.01$ ) than those who received ST (N=35). Ventilatory support was required by 33% of RCE patients, in contrast to 8.6% of ST patients. Hypoxemia was refractory in 66% of RCE patients, in contrast to 31% in ST recipients. Interestingly, the mean change in CRS from pre- to post-transfusion was not different between those who received ST vs RCE (-1.3 vs -1.4,  $p = 0.84$ ).

**Conclusion:** Based on our results, both methods appear to be effective in treating ACS. Though the rate of CRS improvement was similar in both groups, patients who received RCE tended to be more critically ill, with higher pre-transfusion CRS, increased requirement of ventilatory support, more refractory hypoxemia, and thus, longer hospital stay. CRS did not predict which patients would fail to respond to ST ( $p=0.13$ ), but CRS reliably predicted which patients would have refractory hypoxemia and longer hospital stay ( $p<0.01$ ). In summary, CRS predicted which patients would have poorer outcome, but could not predict who would fail ST and thus benefit from RCE.

[1] Saylor, *Pediatr Blood Cancer*, 2013.

Poster # 535

## **DEFECTS IN OSTEO-CHONDROGENIC LINEAGE COMMITMENT IN MOUSE MODELS OF DIAMOND BLACKFAN ANEMIA: POTENTIAL ROLE IN ONCOGENESIS**

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**Background:** Osteogenic sarcoma (OS) is the most common primary bone malignancy in children and young adults. These histologically variable tumors are characterized by the presence of osteoid and osteoblast-like cells. Patients with Diamond Blackfan anemia (DBA), an inherited bone marrow failure syndrome characterized by red cell aplasia and congenital anomalies, are at an increased risk of developing OS with an observed-to-expected ratio of 32.6. The majority of DBA cases arise from ribosomal protein gene haploinsufficiency, which leads to defective ribosome synthesis and nucleolar stress.

**Objectives:** In the present study, we aimed at investigating how ribosomal protein gene haploinsufficiency affects osteoblast differentiation leading to the development of OS.

**Design/Method:** We studied osteoblastogenesis *in vitro* using murine embryonic stem (mES) cells gene trapped for one *Rps19* or *Rpl5* gene. mES cells were differentiated to osteoblasts for 10 days in the presence of ascorbic acid, 1,25-dihydroxyvitamin D, dexamethasone and  $\beta$ -glycerophosphate. Cultures were characterized morphologically using a series of histologic stains including alizarin red, alkaline phosphatase and alcian blue. Levels of key transcription factors for osteoblastogenesis and chondrogenesis were assessed by qRT-PCR and western blot analyses.

**Results:** Alizarin red and alkaline phosphatase staining revealed that mineralization was attenuated in both models of ribosomal haploinsufficiency, but only *Rpl5*<sup>+/-</sup> cells exhibited a growth defect. Moreover, cartilage matrix production was increased (10- and 2-fold in *Rps19*<sup>+/-</sup> and *Rpl5*<sup>+/-</sup>, respectively) suggesting biased lineage determination between bone and cartilage-forming cells. To confirm this, we investigated the expression of key transcription factors

involved in osteoblast (*e.g. Runx2*) and chondrocyte differentiation (*e.g. Sox6* and *Sox9*) throughout osteogenesis. Compared to wild-type cells, *Rps19*<sup>+/-</sup> cells expressed decreased RUNX2 and elevated levels of SOX6 and SOX9 consistent with increased alcian blue staining. Conversely, RUNX2, SOX6, and SOX9 expression was unchanged between wild type and *Rpl5*<sup>+/-</sup> cells.

**Conclusion:** Together, these data indicate that ribosomal biogenesis is essential for osteoblast development and that ribosomal gene haploinsufficiency differentially affects the transcription networks regulating osteochondrogenic lineages. Therefore, defective osteoblast differentiation downstream of ribosomal haploinsufficiency may be an important step in the formation of DBA-associated OS.

Poster # 536

## **THE EARLY IMPLEMENTATION OF HYDROXYUREA IN SICKLE CELL DISEASE PATIENTS WITH CONDITIONALLY ABNORMAL TRANSCRANIAL DOPPLER ULTRASOUNDS TO DECREASE ADVERSE SEQUELAE AND PREVENT NEED FOR CHRONIC TRANSFUSIONS**

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**Background:** Sickle Cell Disease (SCD) patient are at risk for multiple life threatening sequelae including stroke. The risk for stroke is quantified with a transcranial Doppler (TCD) ultrasound. Values >200 cm/sec are considered high risk for stroke, 170-200 cm/sec a conditionally high risk, and <170 cm/sec is normal. Current management is regular, chronic RBC transfusion for those with TCD >200 cm/sec, however studies show that despite this intervention, a substantial proportion of patients continue to have an increased risk for stroke. Hydroxyurea (HU), a potent chemotherapeutic agent, has been proven to decrease several complications of SCD in adult patients.

**Objectives:** We aimed to study if early implementation of HU in SCD patients with conditionally abnormal TCDs could abrogate the need for chronic transfusions, and subsequent neurological sequelae.

**Design/Method:** A retrospective chart review of the Southern California Permanente Medical Group sickle cell data registry of patients aged 0-18 who had conditionally abnormal TCDs and started on HU between 01/01/2006 -10/30/14 was conducted. Exclusion criteria included those who were on chronic transfusions and/or had a history of receiving transfusions. We applied a sensitivity analysis model to analyze the data both with and without outliers of patient response.

**Results:** A total of 35 patients were identified. 6 were excluded due to simultaneous RBC transfusions. 7/29 were started on HU for a reason other than a conditionally abnormal TCD. 19/29 had improvement or stabilization of their TCDs with mean follow-up of 979 days, 4/29 had a <20 cm/sec increase in their TCD, and 5 had progression into the abnormal TCD category.

**Conclusion:** In our small cohort, early implementation of HU improved or stabilized TCDs in a majority of conditionally high risk sickle cell patients. Non-compliance was the single most significant factor that contributed to increasing TCDs. Preliminary results from a non-inferiority trial (TWiTCH) with HU in SCD patients with high risk TCDs supports the notion that HU may have a therapeutic benefit.

Hoots, Ware, Davis, NIH, 2014.



Poster # 537

## **DELETERIOUS CONSEQUENCES OF DIAMOND BLACKFAN ANEMIA (DBA) ON REPRODUCTIVE HEALTH AND PREGNANCY OUTCOMES: A REPORT FROM THE DIAMOND BLACKFAN ANEMIA REGISTRY (DBAR)**

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**Background:** DBA is a rare, congenital bone marrow failure syndrome characterized by red cell aplasia, birth anomalies, and a predisposition to cancer. Available treatment includes corticosteroid administration, chronic red cell transfusion therapy and stem cell transplantation. Clinical observations suggest that women with DBA experience delayed puberty, irregular menstrual cycles, and decreased fertility. Women may sustain a higher risk of pregnancy complications, including miscarriage, placental abruption, and stillbirth. Anecdotal reports suggest that DBA women experience changes in steroid treatment responses and remission status during puberty and pregnancy.

**Objectives:** To document gynecologic and pregnancy outcomes and complications in these women and to correlate these to their treatments.

**Design/Method:** Questionnaires inquiring about menstrual and gynecologic health and pregnancies were sent to females over 15 years of age participating in the DBAR . We grouped patients by steroid and/or transfusion dependence status and/or remission. The Fisher's exact test was used to examine associations between variables.

**Results:** Eighty four women, ages 15 to 62 years (median 28.6 years) completed the questionnaire. Menarche was delayed in both steroid dependent (SD) and transfusion dependent (TD) girls, with 39.5% and 77.8%, respectively, experiencing menarche at age 15 or later. TD girls were significantly more likely to have delayed menarche compared to the SD and remission groups ( $p < .005$ ). Additionally, TD females were significantly more likely to experience premature ovarian failure (POI), compared to those who were either SD or in remission (32.1% vs 8.6%;  $p < .025$ ). 75% of those who entered menopause did so by age 40. Of the 50 pregnancies in 23 women, 30 resulted in live births (60%); 43.3% were preterm. SD women and those in remission had the highest percentage of preterm deliveries at 66.7% and 63.6%, respectively. 26% of the all pregnancies resulted in miscarriage, 4.3% in stillbirth and 4.3% were complicated by placental abruption. 100% of SD women required transfusions during pregnancy.

**Conclusion:** These findings suggest an important role of iron overload in menstrual abnormalities, reproductive issues and endocrinopathies in DBA women. Pregnancy complications are in excess of those seen in the general population. Future analyses may determine a correlation between these complications and specific DBA genotypes.

Poster # 538

## **BARRIERS TO IMPROVING ANALGESIA FOR VASO-OCCLUSIVE PAIN CRISES IN PEDIATRIC PATIENTS WITH SICKLE CELL DISEASE**

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**Background:** Vaso-occlusive pain crises (VOC) are the most common cause of morbidity and hospitalization in patients with sickle cell disease (SCD). Despite evidence of patient-controlled analgesia (PCA) safety and efficacy in children with VOC, there is wide variation in provider comfort level prescribing narcotics and PCA.

**Objectives:** This quality improvement project aims to assess the current need for improvement in analgesia for children with SCD admitted from the Pediatric Emergency Department (PED) for VOC, to evaluate provider comfort prescribing narcotics for pediatric patients with VOC, and to identify barriers to early PCA initiation.

**Design/Method:** The records of pediatric patients with SCD who were admitted for VOC from Duke University Medical Center PED during October 2013-March 2014 were reviewed. Patients < 18 years or followed by Pediatric Hematology service, with SCD, type SS, SC, or S plus thalassemia were included. An in-service lecture was given to pediatric residents and medical students after the 6 month retrospective chart review period. A questionnaire regarding experience/knowledge and perceptions regarding PCA was administered prior to and following the lecture. Descriptive statistics are reported.

**Results:** Nineteen hospitalizations met criteria for inclusion. Median age was 13.4 years (range 3.6-18.2). Patients received a median 3 (range 0-5) intermittent narcotic doses prior to admission. PCA was begun in only 2 patients while in PED, and in 15 while inpatient. Median length of hospitalization was 6.5 days (range 1-11) for those who began PCA as inpatients.

Twenty-eight pre- and 27 post-lecture questionnaires were completed. Seventy-one percent of respondents had never ordered, and 21% had ordered PCA 1-5 times in the PED. Barriers to PCA initiation included "time constraints" (79%), prescriber discomfort/inexperience (79%), and patient age (46%). Improved comfort with PCA use following the in-service was reported in 81%, and increased likelihood of PCA initiation in the PED for VOC in 89%.

**Conclusion:** PCA is infrequently initiated for pediatric patients with VOC in the Duke PED. House staff report limited experience prescribing narcotics in this population. This study shows that there is a clear unmet need for focused education for housestaff on pain management. Pain control for VOC in children remains an area for improvement across disciplines.

Poster # 539

## **GENOTYPE-PHENOTYPE CORRELATION OF PHYSICAL ABNORMALITIES IN PATIENTS WITH FANCONI ANEMIA**

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**Background:** Physical anomalies are known to occur in children with Fanconi Anemia, but relationship to genotype and laterality/symmetry of anomalies have not been systematically explored.

**Objectives:** We systematically described anomalies in a large population of children with FA.

**Design/Method:** Physical examination and data extraction was performed by experienced clinicians.

**Results:** Eighty one patients were evaluated (39 male).

Upper limb anomalies were commonly bilateral (n=37). However, when unilateral, both upper limb (12/17, p=0.06) and renal anomalies (13/21, p= NS) favored a left sided predominance.

Interestingly, 28% and 57% of patients did not show anomalies of the upper limbs or skin pigmentation changes, respectively. Importantly 8 patients had no physical signs associated with FA suggesting need for a high index of suspicion.

**Table 1** lists anomalies in 55 patients with an available genotype. Upper limb abnormalities were seen in all genotypes except FANCP (n=1). Interestingly, a significant number of children (49/55) did not have the typical radial ray anomalies described in FA. Similarly, majority of children with FANCA showed isolated thumb anomalies without a combined thumb-radial anomaly.

A significant number of clinically important abnormalities involving the GI tract, CNS and vertebral system were seen, highlighting the importance of comprehensive screening of these high risk patients.

**Conclusion:** Physical anomalies in patients with FA occur on both sides, though are commonly asymmetrical. Anomalies are also seen in organ systems not routinely evaluated. The increased availability of genetic testing will allow further genotype-phenotype correlation and improved understanding of organogenesis and laterality in these patients.

Fanconi Gene Mutated (n=55)												
Abnormality	A (n=28)	C (n=10)	L (n=2)	J (n=2)	F (n=1)	I (n=2)	D2 (n=2)	G (n=2)	D1 (n=2)	B (n=2)	E (n=1)	P (n=1)
<b>Hand (n=41/55)</b>												
-Thumb	9	4				1	1	2	1	1	1	
-Radial + thumb			1	2						1	1	1
-Thenar eminence	13	2	1									
<b>Foot (n=5/55)</b>												
-Bony abnormality	2		1									
-Syndactyly	3	1										
<b>Café-au-lait/hypopigmentation (n=24/55)</b>												
-Low set ears	2	2					2					
-Pinna	1		1							1	1	
-Canal hypoplasia	3			2							1	
-Absent meatus				1								
-Hearing loss	4	2	1	1								1
<b>Renal (n=20/55)</b>												
-Ectopic/fused	4	2			1				2	1	1	
-Atrophic	1	1										
-Single kidney				1								
-Horseshoe	2											
-Dysplastic/cystic	1						1					
<b>Other (n=25/55)</b>												
-GI	5	1		2					1			
-Cardiac	4	1		1			1		1	1		
-Microcephaly	6			1					2		1	1
-Vertebral	4	2							1		1	
-Chiari	3	1										
-Malformation												
-Hydrocephalus				1								
-Small optic nerves							1					
-Facial Droop	1											
-Tethered cord		1							1		1	
-Microphthalmia	1											
-Respiratory	1											
-Hypospadias				1								

Poster # 540

## NO MEAN FEAT: PREDICTING RECURRENT SPLENIC SEQUESTRATION IN PEDIATRIC SICKLE CELL PATIENTS

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**Background:** Splenic sequestration (SPLSEQ), a common, sometimes life-threatening complication of sickle cell anemia (SCA) occurs predominantly in children aged below 5 years. Recurrent SPLSEQ occurs in approximately 50% of first episode survivors.

**Objectives:** To identify predictors of recurrent SPLSEQ to guide therapeutic choices for children at highest risk.

**Design/Method:** Retrospective chart review of 38 SCA patients with SPLSEQ (definition: acute drop in hemoglobin  $>2$  g/dL below baseline with appropriate reticulocytosis and splenomegaly) was performed after the IRB granted a waiver of consent. A case control study design was utilized; cases were children with SCA and 2 or more episodes of SPLSEQ; controls were age-matched SCA patients with 1 such episode. Demographic and hematologic data was collected and compared between groups, number of transfusions required per episode and history of surgical splenectomy. Timepoint selected for data collection was the last known steady state value obtained at a clinic visit before the first episode of SPLSEQ for both groups. Steady state was defined as no transfusions within two months and no acute SCA events within one month. Absolute T test comparisons were used to determine differences between the 2 groups.

**Results:** 38 patients (21 males, 17 females) were included (19 per group). Mean age in years at first episode did not differ between cases and controls ( $2.3 \pm 2.4$  years vs.  $2 \pm 1.4$  years,  $p=0.67$ ) nor did number of transfusions per episode ( $1.5 \pm 0.4$  vs.  $2 \pm 1$ ,  $p=0.15$ ). 8/19 (42.1%) of cases underwent surgical splenectomy, none amongst controls. 2/38 patients (1 case, 1 control) had HBS $\beta$ 0 thalassemia; rest had HbSS. No statistical differences in steady state pre-SPLSEQ hematologic parameters were noted between the cases and controls respectively at the selected timepoints (WBC  $10.4 \pm 4.6$  vs.  $9.9 \pm 3.04$  K/mcl,  $p=0.71$ ; ARC or Absolute Reticulocyte Count  $277.3 \pm 155.8$  vs.  $221.5 \pm 110.7$  K/mcl,  $p=0.22$ ; Hemoglobin  $8.3 \pm 1.2$  vs  $8.6 \pm 1.3$ g/dl,  $p=0.46$ ).

**Conclusion:** Not surprisingly more cases with recurrent SPLSEQ underwent surgical splenectomy. In this cohort of patients no statistically significant differences were noted in any hematological parameters between patients with and without recurrent SPLSEQ. Larger studies are needed to determine recurrence predictors to improve treatment.

Poster # 541

## REPLICATIVE STRESS LEADS TO PRENATAL STEM CELL ATTRITION IN FANCONI ANEMIA

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**Background:** Bone marrow failure is the leading cause of morbidity and mortality in Fanconi Anemia (FA). FA hematopoietic stem and progenitor cells (HSPC) are vulnerable to experimental genotoxic stress by alkylating agents, aldehydes, and cytokine exposure; however, developmental deficits and hematopoietic failure in FA have origins *in utero* suggesting a physiological basis for stem cell attrition during development.

**Objectives:** FA proteins are critical for coping with the physiologic DNA damage accrued during fetal self-renewal divisions in the rapidly expanding hematopoietic stem cell pool.

**Design/Method:** We harvested fetal livers (FL) from mice with *Fancc* and *Fancd2* disruption at embryonic day 14.5 and investigated HSPC content and function, DNA damage response, and the *in vitro* and *in vivo* reversal of observed deficits with pharmacologic inhibition of a key stress kinase pathway.

**Results:** The long term repopulating ability of *Fancd2*-deficient FL is significantly compromised with 61% fewer SLAM-HSC and a decrease in engraftment of 73% in serial transplantation assays. There is broad transcriptional upregulation of genes involved in DNA damage response and repair and a significant increase in nuclear RAD51 foci, a protein involved in stalled replication fork restart. Micro-RNA(miR)-125b is significantly downregulated in *Fancd2*<sup>-/-</sup> FL with downstream activation of *p21*, both consistent with known stress activation of *p53*.

However, these cells are undergoing neither apoptosis nor senescence based on transcriptional gene expression profiling of *Puma*, *Noxa*, *p15*, and *p16*. HSC functional deficits in *Fancd2*<sup>-/-</sup> FL are ameliorated by pharmacologic inhibition of stress-induced p38 MAP kinase, demonstrated by a 120% increase in *in vitro* colony formation and a 100% increase in donor chimerism following transplantation.

**Conclusion:** We demonstrate the physiological role of FA proteins in protecting rapidly dividing fetal HSPC from accruing DNA damage and activating a cell stress response pathway. We propose a model whereby replication induced cell stress in FA deficient fetal HSPC promotes activation of downstream stress responders, including the p38 MAPK pathway and *p21*, with coordinate suppression of key self-renewal regulator, miR-125b. Our findings confirm that the HSPC pool in FA is compromised well before birth, and that FA proteins play an important physiological role during development.

Poster # 542

## **PROGRESSION OF CEREBRAL VASCULOPATHY IN CHILDREN WITH SICKLE CELL DISEASE UNDERGOING CHRONIC TRANSFUSION THERAPY FOR PRIMARY AND SECONDARY STROKE PREVENTION**

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**Background:** Acute and silent cerebral infarcts are significant morbidities in patients with sickle cell disease (SCD). Patients with Hgb SS have an 11% chance of a first overt stroke by age 20.<sup>1</sup> The prevalence of silent stroke is higher at 33%.<sup>2</sup> Chronic transfusion therapy decreases both the risk of first stroke in patients with abnormal transcranial Doppler screens and the risk of recurrence in patients with previous infarct.<sup>3,4</sup> Recent studies using magnetic resonance imaging and angiogram (MRI/MRA) suggest that a subset of patients may have progression of cerebrovascular disease despite chronic transfusions.<sup>5,6</sup>

**Objectives:** Examine the frequency of progression of cerebral vasculopathy in children undergoing chronic transfusion therapy for both primary and secondary stroke prevention and investigate the similarities in those whose vasculopathy progresses despite transfusion.

**Design/Method:** We performed a retrospective chart review of 38 children with SCD (average age 15.50 years, *SD* = 4.59) on chronic transfusion therapy (average duration of therapy 105.16 months, *SD* = 60.28.) for primary or secondary stroke prevention. Demographic information collected included gender, current age, the reason for transfusion therapy (stroke, TIA, abnormal TCD) and age initiation, total lifetime packed red cell units received, and average annual % hemoglobin S and ferritin levels. Imaging results were prospectively (re)reviewed by a single radiologist to assess changes in vasculopathy over time.

**Results:** Preliminary data shows patients who were started on therapy for secondary stroke prevention were more likely to have progression (21%) than patients started for primary prevention (7%). Patients who were started on transfusion for primary stroke prevention were typically younger (mean 5.07 years) than those receiving secondary prevention (mean 7.6 years). Despite poorer outcomes for patients on therapy for secondary prevention, three patients on therapy due to prior infarcts evidenced resolution or improvement in vasculopathy. Children on therapy for primary prevention were less likely to have abnormal vasculopathy at initiation of transfusion therapy (15% vs. 71% of patients).

**Conclusion:** Early detection of stroke risk in children with SCD is essential as therapy can

improve or prevent progression of vasculopathy. Larger, multi-institutional studies are needed to better identify risk factors for progression of vasculopathy despite transfusion therapy.

Poster # 543

### NEUTROPENIA IN INFANCY: A NORMAL PROCESS?

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**Background:** Reference ranges in children are difficult to obtain and many laboratories use published reference ranges from the literature, most of which have not been developed according to laboratory standards for their development. Neutropenia in infants and children is often a source of concern for both parents and clinicians. The medical literature is varied with some descriptions of a benign neutropenia of childhood and others an auto-immune neutropenia of infancy being a serious condition, associated with severe infections and significant mortality.

**Objectives:** We sought to identify the frequency and natural history of neutropenia in a population of infants presenting to a tertiary care centre.

**Design/Method:** Complete blood counts were reviewed from all children 1 year of age (365 days) and under, done in a tertiary care children's hospital from April 1<sup>st</sup>, 2012 through March 30<sup>th</sup>, 2013. The frequency of neutropenia, defined as neutrophil count less than  $1.0 \times 10^9/L$  and natural history over the year as well as the clinical implications were reviewed.

**Results:** Two thousand and sixty-seven children had a total of 5977 CBCs under age 1, with each infant having between 1 and 144 blood counts during the year studied. Two hundred and forty nine infants (12%) had at least one episode of neutropenia, with a total of 587 neutropenic CBCs (9.8% of CBCs). Forty percent of the neutropenic CBCs had severe neutropenia with counts less than  $0.5 \times 10^9/L$ . There were two peaks in the age at the time of neutropenia, one in the second month of life and the other at 6 months. There was resolution of neutropenia documented in over 2/3 of the patients who had repeat blood counts at our institution.

**Conclusion:** Neutropenia, including severe neutropenia is not uncommon in infants less than one year of age. The majority resolve spontaneously without being the cause of serious bacterial infection.

Poster # 544

### SIGNIFICANT VARIATION IN SERUM CREATININE LEVELS AMONG PEDIATRIC PATIENTS WITH DIFFERENT SICKLE CELL GENOTYPES

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**Background:** Kidney damage due to sickle cell disease (SCD) begins in the first decade of life. Serum creatinine (SCr) is a poor indicator of estimated glomerular filtration rate (GFR) in young patients with SCD due to hyperfiltration, increased tubular secretion, and lower muscle mass frequently found in this population. Generally, it is accepted that SCr is lower than normal in SCD, but differences in SCr among the different SCD genotypes are not well-established.

**Objectives:** To determine the association of SCr with genotype in a cohort of pediatric SCD

patients.

**Design/Method:** Electronic medical records for patients with SCD who sought care at the Children's Hospital of Richmond Comprehensive Sickle Cell Clinic between January 1, 2011 and December 31, 2013 were retrospectively reviewed after consent was obtained by their caregivers. Genotypes were confirmed by chart review and hemoglobin electrophoresis. Hemoglobin levels and SCr were averaged from ambulatory, emergency, and hospital visits over the past 12 months from time of enrollment. The Mann-Whitney U test was used to compare SCr and hemoglobin levels between SCD genotypes. Spearman's rho was used to correlate age, SCr, and hemoglobin variables.

**Results:** There were 125 SCD subjects enrolled: 77 (61.6%) HbSS, 32 (25.6%) HbSC, 12 (9.6%) HbS $\beta^0$ -thalassemia, and 4 (3.2%) HbS $\beta^+$ -thalassemia. Median age was 10 years (range 1-17). Median SCr in HbSS subjects was 0.34mg/dl (0.18- 1.20) versus 0.53mg/dL (0.24-0.82) for HbSC patients ( $p<0.001$ ). As expected, median hemoglobin levels were also significantly different between these two groups: 8.5g/dL (5.9-11.1) in the HbSS group and 10.8g/dL (9.2-14.7) in the HbSC group ( $p<0.001$ ). Age distribution was not different between the two groups ( $p=0.402$ ). Age and SCr were positively correlated among both genotypes: HbSS ( $r_s=0.71$ ;  $p<0.001$ ), HbSC ( $r_s=0.61$ ;  $p<0.001$ ). A significant positive correlation between SCr and hemoglobin was found in the HbSC cohort ( $r_s=0.53$ ;  $p=0.002$ ) but no significant correlation was demonstrated in the HbSS group ( $r_s=0.17$ ;  $p=0.13$ ).

**Conclusion:** SCr is significantly lower in pediatric HbSS patients versus those with HbSC, suggesting a greater degree of hyperfiltration and early renal damage in this group. Our findings demonstrate the need for further research of markers of sickle nephropathy in young patients with various SCD genotypes.

Poster # 545

## IDENTIFICATION AND CLINICAL CHARACTERIZATION OF CHILDREN WITH BENIGN NEUTROPENIA

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**Background:** Benign neutropenia (BN) is an asymptomatic condition observed in individuals of African descent. Patients with BN tend to have normal myeloid maturation but release fewer neutrophils into the peripheral circulation. BN has been previously reported to have a male predilection with no reported increased risk of infection.

**Objectives:** The purpose of this study is to more fully characterize the clinical findings seen in patients with BN.

**Design/Method:** A retrospective study was conducted at an urban tertiary care children's hospital in Washington, DC after the institutional IRB granted a waiver of consent. Patients were identified by searching ICD9 codes over a 5 year period (2008-2013) for neutropenia and then demographic, laboratory, and clinical information was reviewed for eligibility.

**Results:** One hundred thirty-six patients were identified with neutropenia. Thirty-seven (27.2%) were classified as BN. The remaining, ninety-nine (72.8%) were excluded because of known causes of neutropenia [autoimmune (43), self-resolved neutropenia (26), malignancy (22), or other (8)]. The median age of the BN cohort was 5.3 years; nineteen were males and eighteen were females. Twenty-two (59.5%) patients were of African descent, four (10.8%) Caucasian, three (8.1%) Hispanic, two (5.4%) Asian. Median ANC was  $893 \times 10^6$  cells/L. The remainder of

the complete blood count as well as immunoglobulins were all within normal range for age. No first degree relatives of any of the patients were noted to have a history of neutropenia. Five BN patients (13.9%) required hospitalization; two for febrile neutropenia, one each for mastoiditis, pneumonia, and weight loss with neutropenia. None of these hospitalizations required ICU management. Five patients had bone marrow biopsies, three of which were normal and two had focal/mild hypocellularity.

**Conclusion:** Compared with patients with congenital neutropenia, few BN patients were hospitalized. Sixty percent of our BN cohorts were of African descent and 24% were of other ethnic backgrounds. Nearly half were female. Further studies are necessary to elucidate the underlying molecular genetics underlying this condition.

Poster # 546

## **INITIAL VALIDATION OF A RAPID PAPER-BASED TEST IN SCREENING NEWBORNS FOR SICKLE CELL DISEASE**

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**Background:** Newborn screening for sickle cell disease (SCD) in developing countries is limited the cost and technical complexity of current screening methodologies and the delayed availability of screening results. We have recently developed a diagnostic test for SCD that can quickly and inexpensively identify blood samples containing hemoglobin S.

**Objectives:** The objective of this project was to determine the feasibility and accuracy of the rapid SCD test as a screening tool at the newborn screening program in Cabinda, Angola.

**Design/Method:** After informed consent, blood samples were collected by heel-stick from neonates and by finger-stick from mothers at the primary obstetric hospital in Cabinda. These samples were then subjected to the rapid SCD diagnostic test and scored independently by visual assessment of staining patterns. Neonates and mothers were scored as positive (HbS detected) or negative (no HbS detected). Infant samples were subsequently tested by isoelectric focusing (IEF) electrophoresis. The sensitivity and specificity of the maternal and neonatal rapid tests in identifying neonates with SCD were determined by comparing rapid test results to IEF results.

**Results:** Our initial testing cohort consisted of 32 infant-mother pairs. Testing of mothers with the rapid diagnostic test indicated that 26 (81.2%) had the AA genotype, 6 (18.8%) had the AS genotype, and none had the SS genotype. Among the neonatal samples, 21 (65.6%) were negative and 11 (34.4%) positive for HbS. Subsequent IEF testing of all 32 neonatal samples revealed that 27 (84.4%) were AA, 4 (12.5%) were AS, and one (3.1%) was SS. The sensitivity and specificity of the maternal rapid test relative to IEF for identification of affected neonates were 1 and 0.84 respectively, while those for the neonatal test were 1 and 0.68.

**Conclusion:** The maternal and neonatal rapid tests were both highly sensitive for identifying newborns with SCD, indicating their potential utility as a screening tool prior to more definitive testing. Further validation of these initial results with larger numbers of patient samples is currently underway. Used in combination with confirmatory IEF, our rapid test could significantly decrease the cost of newborn screening for SCD and increase its clinical utility by permitting more rapid identification of affected infants.



## MULTIDISCIPLINARY CARE FOR COMPLEX DISORDER: SHWACHMAN-DIAMOND SYNDROME (SDBS) AS A SINGLE-CENTER EXPERIENCE AND EXAMPLE

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**Background:** SDBS is a rare autosomal disorder initially described as a combination of neutropenia and pancreatic insufficiency. SDBS patients may present other anomalies including neurocognitive impairment, increased risk of infection and MDS/leukemia. Care of SDBS is therefore complex and requires multidisciplinary approach.

**Objectives:** Describe single-center SDBS cohort.

**Design/Method:** Retrospective chart review of patients followed at multi-disciplinary SDBS clinic, developed in 2011 within our inherited marrow failure program, a team composed of nutritionist, haematologist, gastroenterologist and nurse.

**Results:** Twelve patients have been followed (see table), with a current median age of 8.9 yrs. (4.8-19.1). Diagnosis was established upon clinical criteria. SDBS mutations were searched in 8/12 patients: compound heterozygosity for a c.258+2TC mutation was identified in all, either with c.120delG or c.183\_184TA>CT. Enzyme replacement is required in all but two patients to ensure regular growth. All but one patient are alive; one had been transferred to an adult haematologist. 4/12 developed severe haematological anomalies requiring HSCT: two for severe pancytopenia, one for AML at 5.2 yrs. (the youngest SDBS with AML to our knowledge), and one for high percentage of cytogenetic anomaly. Engraftment failure occurred in three patients, particularly when cord blood was used. One patient had acute GVHD. One died of infectious complications post-HSCT.

**Conclusion:** SDBS is a severe disorder requiring multidisciplinary care. Our experience confirms natural evolution toward complex haematological anomalies at high rate. In case of HSCT cord blood should not be a first stem cell source, as it may be associated with higher risk of engraftment failure.

Pt	M/F	Age dg	Length f-up	Genetic	HSCT	Current Status
1	F	7.3	4.2	c.258+2TC / c.120delG	None	Well
2	M	0.2	11.0	n.d	None	Well
3	M	1.0	6.1	c.258+2TC / c.120delG, c.120delG	3 HSCT for AML	CR, 14mo post HSCT
4	M	1.0	3.8	c.258+2TC / c.120delG, c.120delG	None	Well
5	F	3.4	12.6	n.d	None	Well
6	M	0.5	9.2	c.183_184TA>CT / c.258+2T>	None	Well
7	F	3.4	4.8	c.183_184TA>CT / c.258+2T>	HSCT for pancytopenia	CR, 5mo post HSCT
8	M	1.0	9.5	c.258+2T>C / c.183_184, TA>CT	HSCT for clonal hematopoiesis	Died - BMT related mortality
9	F	1.1	7.7	c.183_184TA>CT / c.258+2T>	None	Well
10	M	2.6	4.0	c.258+2T>C / c.183-184delinsCT	None	Well
11	M	5.8	14.5	n.d	None	Transfer adult Hematologist
12	M	2.5	16.6	n.d	2 HSCT for pancytopenia	CR, 10 yrs post HSCT

## RED BLOOD CELL ALLOIMMUNIZATION IN SICKLE CELL DISEASE IN THE ERA OF EXTENDED RED CELL TYPING: A SINGLE-CENTER EXPERIENCE

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**Background:** Transfusion therapy is a key intervention in decreasing morbidity and mortality in patients with sickle cell disease (SCD). The development of antibodies to red cell antigens remains a major complication associated with RBC transfusions in patients with SCD. It can make finding compatible blood for transfusion more difficult, expensive and time consuming. A number of studies demonstrate that extended phenotype RBC matching can minimize alloimmunization.

**Objectives:** The aim of this study was to report our experience with extended RBC antigen matching for transfusions in SCD patients.

**Design/Method:** Since 1997, the institutional policy for transfusion of patients with SCD has been to antigen match prospectively for D, C, E, and K antigens; patients whose RBCs lack the antigen are transfused with antigen-negative donor units. This study is a 6-year retrospective analysis (2009-2014) of patients with SCD at our Comprehensive Sickle Cell Center, transfused with RBCs that were prospectively matched for D, C, E, and Kell and were primarily from African American donors.

**Results:** During the study period, 89 patients (48 females and 41 males), aged 6-25 years (mean 15.2) underwent RBC phenotyping for 18 blood group antigens and 1459 RBC units that were matched for D, C, E, and Kell were provided. Alloimmunization incidence was 15.7%. A total of 37 alloantibodies were detected in 14 patients (23 new and 14 preexisting alloantibodies). Those who developed alloantibodies were older (15.2 versus 11.2 years;  $p < 0.01$ ) and had a higher number of transfusions (35.1 vs 11.3 units;  $p < 0.01$ ). The most common detected alloantibodies were Rh-related. RhC, E alloimmunized patients were more likely to develop antibodies against other antigens.

**Conclusion:** The major findings of our study are (1) The alloimmunization rates continue to be significant even after extended red blood cell antigen matching; (2) Antibodies were predominantly to Rh C and E antigens and antibodies to other low incidence antigens were not significantly increased. Consideration should be given to exploring this conclusion further with a controlled, multi-institutional trial to determine cost-benefit analysis of extended red cell matching.

Poster # 549

## **DEVELOPMENT OF A NOVEL IN VITRO THREE DIMENTIONAL OSTEOBLASTIC NICHE MODEL TO STUDY HEMATOPOIETIC STEM CELL MIGRATION AND ENGRAFTMENT**

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**Background:** In vitro culture experiments have been the standard platform to study hematopoiesis but traditional liquid cultures do not provide the complex three-dimensional (3D) structure of the bone marrow (BM) niche. The niche provides the hypoxia-gradient for the hematopoietic stem cells (HSCs) to retain their self-renewal. Hence, there is a need for development of culture systems that closely mimic BM physiology and provide a platform for

development of strategies to enhance HSC engraftment and inhibit cancer metastasis.

**Objectives:** Develop a 3D BM niche system that supports migration and lodging of HSCs and utilizes a hypoxia-sensitive intravital reporter to quantify HSC migration/lodgment in the niche.

**Design/Method:** 3D-osteoblastic niche (3D-ObN) spheroids were grown in 96-well ultra-low attachment plates using immortalized human cell lines, *hFOB 1.19* (osteoblasts) in the center, and *HS-5* (fibroblasts) as an outer layer. They were co-cultured with CD34<sup>+</sup> human HSCs stained with a hypoxia-sensitive probe that emits fluorescence only in a hypoxic environment. The cultures were incubated in a live-cell imaging platform that measures fluorescence and captures images in real time during culture.

**Results:** The 3D-ObN spheroids efficiently incorporated human HSCs as evidenced by a time-dependent increase in fluorescence relative to controls of spheroids alone and HSCs alone in culture, neither of which released fluorescence. In a separate experiment, the spheroids also actively recruited T-ALL cells (Jurkat, Malt-4). As a proof-of-principle study, CXCR4 inhibition by adding AMD3100 (Sigma Aldrich) prevented HSC migration to the 3D-ObN in a dose-dependent manner. Interestingly, the combined hFOB/HS-5 spheroids and hFOB-only spheroids actively incorporated HSCs while the HS-5-only spheroids did not, indicating that hFOB cells exert signals for HSC homing. We tested the effects of Celestrol and CDDO-Me (natural and semi-synthetic triterpenoids) on HSC migration to the niche and found that neither enhanced the migration but relatively inhibited it at higher concentrations.

**Conclusion:** Preliminary data showed that our 3D-Ob-Niche model effectively mimics the BM microenvironment that facilitates HSC migration/lodging. This model can serve as a tool for high-throughput screening of molecules that enhance engraftment. This also has the potential to study the competitive occupation of the BM niche by cancer cells in the presence of HSCs.

Poster # 550

## **SERIAL ECHOCARDIOGRAM FINDINGS IN CHILDREN WITH SICKLE CELL DISEASE**

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**Background:** Cardiac abnormalities are well documented in sickle cell disease (SCD); however, there are no longitudinal studies investigating serial changes in cardiac parameters. Since early 1990s, children followed in our Comprehensive Sickle Cell Center have received annual echocardiograms, allowing us to document chronological changes in cardiac abnormalities.

**Objectives:** To determine the age at which abnormalities are first detected on echocardiograms and to describe the clinical correlations in children with SCD.

**Design/Method:** A review of serial echocardiograms performed on 185 children with SCD from 1997 through 2012 was performed. Left heart parameters included LV end-systolic (LVES), end-diastolic (LVED) diameters, LV fractional shortening (LVFS), mass (LVM) and their z-scores. RV pressure was estimated by tricuspid regurgitation gradient (TR). Z-score  $\geq 2$ , LVFS  $< 32\%$  and TR  $\geq 25$  mmHg were considered abnormal. Echocardiographic parameters were correlated with age, oxygen saturation (O<sub>2</sub>), hemoglobin (Hgb), white blood cell count (WBC), platelet count (Plt), total bilirubin (Tbili), and treatment with hydroxyurea (HU).

**Results:** Our population was comprised of 53.0% males, 69.2% with hemoglobin SS, 23.2% hemoglobin SC, and 7.6% hemoglobin  $\beta$ -thalassemia. A total of 938 outpatient echocardiograms

were performed with a mean age of  $6.93 \pm 4.59$  years at first echocardiogram, and an average of  $5.07 \pm 3.32$  studies per patient. Over an average follow-up period of  $6.44 \pm 5.23$  years, abnormal parameters were seen in 26.6%, 43.2%, 57.8%, 32.6% and 32.6% of patients for LVM, LVES, LVED, LVFS and TR, respectively. A subset analysis of echocardiograms performed on 73 patients  $\leq 5$  years of age showed that during that period 11.8%, 27.4%, 30.1%, 12.7% and 14.3% of patients had abnormal LVM, LVES, LVED, LVFS and TR, respectively. Age, WBC, Plt, and Tbili were positively, while Hgb, O<sub>2</sub>, and HU were negatively correlated with cardiac abnormalities.

**Conclusion:** Routine outpatient echocardiograms performed on children with SCD show that up to 30% of patients have developed echocardiogram abnormalities by 5 years of age, lending preliminary evidence for initiation of routine echocardiogram screening by 5 years of age.

Poster # 551

## **TSLP DIFFERENTIALLY TARGETS MULTI-LYMPHOID PROGENITORS AND B LINEAGE CELLS IN NORMAL HUMAN HEMATOPOIESIS**

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**Background:** Overexpression of CRLF2 is associated with Ph-like B-ALL. Normally CRLF2 acts as a component of the receptor activated by the cytokine, TSLP. TSLP increases proliferation of murine B cell precursors, however its role in human B cell production is less clear. Mouse TSLP does not act on human cells thus classic human-mouse xenograft models do not provide TSLP that can activate human CRLF2. Therefore, we engineered immune-deficient NOD/SCID IL-2R $\gamma$  null (NSG) mice to express human TSLP (hTSLP<sup>+</sup> mice), as well as control mice that lack the human TSLP cytokine, (hTSLP<sup>-</sup> mice) thus providing a human TSLP<sup>+</sup>/<sup>-</sup> xenograft model system to study the role of TSLP in normal and malignant hematopoiesis.

**Objectives:** Our goal was to identify the function of TSLP in normal B cell production to provide insights into its potential roles in leukemogenesis and B cell reconstitution after stem cell transplant.

**Design/Method:** hTSLP<sup>+</sup> and hTSLP<sup>-</sup> mice were transplanted with primary CD34<sup>+</sup> umbilical cord blood cells. Mice were euthanized at 5 weeks and bone marrow (BM) harvested for flow cytometry analysis of proliferation (Ki-67 expression), apoptosis (Annexin V and 7-AAD), Bcl2 family prosurvival protein expression and cell counts of developmentally sequential subsets of B lineage cells.

**Results:** B cell production in hTSLP<sup>+</sup> mice was increased by ~3 fold as compared hTSLP<sup>-</sup> mice. TSLP targeted a CD19<sup>-</sup> lymphoid-restricted progenitor population for increased proliferation and expression of Bcl2 family prosurvival proteins, resulting in expansion of the earliest pro-B cells and dramatically altering the ratio of CD34<sup>+</sup> progenitor populations in the BM. The expansion in pro-B cells was maintained by TSLP-mediated increases in survival, but not proliferation, during subsequent stages of differentiation. Analysis of normal pediatric BM samples showed that B cell production in children mirrors progenitor ratios and Bcl2 family expression patterns found in hTSLP<sup>+</sup> but not hTSLP<sup>-</sup> xenograft mice.

**Conclusion:** These data provide evidence that TSLP-induced CRLF2 signals play an important role in vivo in the expansion/survival of normal B cell precursors at different stages of B cell development and provide a foundation for understanding the role of TSLP in leukemogenesis

and B cell reconstitution following stem cell transplant.  
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Poster # 552

## **MISSED OPPORTUNITY? A COMPARISON OF ONLINE COVERAGE OF SICKLE CELL DISEASE AND CYSTIC FIBROSIS**

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**Background:** The recent Ice Bucket Challenge, a social media fundraising campaign for the Amyotrophic Lateral Sclerosis Foundation, suggests that the internet may influence disease focused fundraising and awareness. In the United States, sickle cell disease (SCD) is the most commonly diagnosed monogenetic disease (affecting 1/2,500 births) but has received less research support than other inherited diseases. While cystic fibrosis (CF), another monogenetic disorder, affects significantly fewer Americans (1/3700 US births), CF receives at least eight times greater research funding than SCD. Differences in the use of the internet to raise disease awareness and funds, organize patient groups, and promote research in SCD and CF, however, are largely unexplored.

**Objectives:** To compare the internet presence of SCD and CF using an internet monitoring tool.

**Design/Method:** Mention<sup>®</sup> is an online software tool that monitors and tabulates new internet postings by keyword. Alerts were created for keywords “sickle cell disease”, “sickle cell anemia”, and “cystic fibrosis” alone and in combination with keywords “cure”, “research” and “donate”. Data were collected for 2 weeks in November 2014 which excluded CF and SCD awareness months. Mention<sup>®</sup> culled data from five online sources: Facebook, Twitter, blogs, web, and news. Facebook, Twitter and blogs are described here as “social media”.

**Results:** SCD was 3 times less likely to be mentioned online than CF (2,734 vs. 10,083 posts). Mean daily posts for SCD ( $195.29 \pm 65.97$ ) were significantly lower than for CF ( $720.21 \pm 299.84$ ;  $t(26) = -6.40$ ,  $p < 0.001$ ). The web was the most common source of posts for SCD (40%) while Twitter was the most common source for CF (59%). SCD had fewer social media posts than CF (1,615 vs 7,551), and fewer posts than CF for cure (344 vs 2,124), research (526 vs 1,266) and donate (92 vs 474).

**Conclusion:** SCD had fewer total, average daily, and social media postings than CF.

Discrepancies between SCD and CF posts suggest that the internet may be an under-utilized tool to drive advocacy and fundraising for SCD. The predominance of social media content highlights the internet as a novel tool to increase individual and organizational involvement in research and fundraising efforts for rare diseases.

Poster # 553

## **EDUCATING TRAINEES ABOUT BLOOD PRODUCTS: IMPACT OF A FORMAL TEACHING MODULE IN THE TRANSFUSION SERVICE**

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**Background:** Blood transfusion was the most common procedure performed during hospitalizations in 2011 (1). Pediatric residents often order blood products to treat patients. However, education in transfusion medicine is lacking. We developed a half day, formalized, educational experience in transfusion medicine for all pediatric level-2 (PL-2) residents during their required pediatric hematology rotation, as well as other trainees, such as medical students and Pediatric Hematology/Oncology fellows, who rotate on the Transfusion Medicine service.

**Objectives:** To determine baseline transfusion medical knowledge in trainees. To evaluate the impact of a formal teaching module on increasing transfusion medicine knowledge in trainees

**Design/Method:** Trainees rotating on the Hematology service or the Transfusion Medicine service are given an interactive education session that lasts approximately three hours and takes place in the Transfusion Service. During the session, a Transfusion Medicine attending guides the trainee through operations from initial order placement through dispensing the final blood product. Trainees examine each product and discuss various indications and dosing. Routine testing, such as type and cross, is reviewed. Trainees are administered 10 questions about the basics of transfusion medicine prior to and then immediately after the education session. Questions range from the main components of cryoprecipitate to what to do if a patient is having a transfusion reaction. Average pre- and post-education test scores were compared via the student's t-test.

**Results:** Forty-four trainees have completed the transfusion educational module and completed the pre- and post- session examination. Average pre-test score was 4.5, and average post-test score was 8.8 ( $p < 0.0001$ ).

**Conclusion:** Knowledge of transfusion medicine is essential for safe and effective patient care. In our training program, we incorporated a short transfusion medicine educational module for all pediatric PL-2 residents, Pediatric Hematology/Oncology fellows and other trainees. We demonstrated significant improvement in transfusion medicine knowledge. We hope to expand our module to all trainees at Stanford University School of Medicine and beyond via video module.

1) Agency for Healthcare Research and Quality, Center for Delivery, Organization and Markets, Healthcare Cost and Utilization Project, Nationwide Inpatient Sample, 2011.

Poster # 554

## **COMPARISON OF CLINICAL OUTCOMES BETWEEN ADULT AND PEDIATRIC PATIENTS WITH SICKLE CELL DISEASE (SCD): 3-YEAR FOLLOW-UP IN A PROSPECTIVE, LONGITUDINAL, NONINTERVENTIONAL REGISTRY TRIAL**

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**Background:** Although clinical advances have allowed individuals with SCD to live further into adulthood, treatment remains challenging due to increased rates of complications.

**Objectives:** To characterize the disease, complications, and treatment patterns of pediatric and adult patients with SCD, we conducted a prospective registry study.

**Design/Method:** Patients  $\geq 2$  years old with HbSS, HbSC, or HbS/ $\beta$ -thalassemia SCD were enrolled from 57 US centers. Differences between pediatric and adult patients were assessed

every 6 months until 3 years.

**Results:** At baseline, among 498 included patients (317 pediatric; 181 adults), pediatric patients had more asthma/airway reactive disease, dactylitis, and splenic sequestration (Table). Adults had significantly poorer performance status ( $P<0.0001$ ) and more avascular necrosis, gallbladder disease, leg ulcers, and pulmonary hypertension. The most common SCD crisis was pain, both in the 5 years prior to study (86.2% of adults, 72.2% of children) and during study (64.1% of adults, 69.4% of children). During study, more pediatric patients had respiratory conditions, including acute chest syndrome (ACS). More than half of all patients were hospitalized; key reasons were pain, fever, and ACS. Significantly more pediatric patients were hospitalized due to fever ( $P<0.0001$ ) and significantly more adult patients were hospitalized for transfusion/chelation ( $P=0.0336$ ). Absenteeism from school/work was frequent prior to and during study.

**Conclusion:** SCD is associated with significant complications contributing to high hospitalization and absenteeism rates. However, patterns of disease and complications differed between children and adults. Limitations included observational study design, variation in time from diagnosis, and lack of mandatory data collection.

Poster # 555

## EFFECTIVENESS OF PLATELET TRANSFUSION GUIDELINES IN A PEDIATRIC TERTIARY CARE SETTING

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**Background:** Transfusion guidelines help to ensure that utilization of blood products is appropriate. Evidence-based pediatric transfusion guidelines were introduced at our institution in July 2013. The content includes appropriate indication, dosing, and transfusion thresholds.

**Objectives:** To ascertain the impact of guideline introduction on the appropriate utilization of platelets (PLT) at our institution.

**Design/Method:** PLT transfusion indication, volume transfused, related transfusion thresholds and associated clinical information were assessed for two different time periods: six-months immediately prior to (Jan-Jun 2013)[PRE] and in a six-month period following full transfusion guideline implementation (Dec 2013-May 2014) [POST].

**Results:** Patients with hematologic malignancies were the greatest users of PLTs in both study periods (Table I). The acute lymphocytic leukemia group was transfused at a median PLT count of  $18 \times 10^9/L$  (range 1-83) [PRE] compared with 19 (range 5-382) [POST] ( $p = 0.10$ ). For the acute myeloblastic leukemia group, the median PLT count was 15 (range 4-58)[PRE] and 20 (range 1-84) [POST] ( $p = .004$ ). An evaluation of transfusions that occurred with a PLT count  $> 100$  showed that the majority of these occur in the OR or intensive care unit (ICU) settings.

**Conclusion:** The implementation of guidelines has not appeared to have a significant impact on PLT transfusion thresholds. Patients with hematologic malignancy were transfused appropriately. However, there were a small number of PLT transfusions that occurred at counts  $>100 \times 10^9/L$  and likely reflects patient complexity found in the OR/ICU settings. These locations may benefit from point-of-care instruments that measure PLT count and function to better guide platelet therapy.

Table I. Characteristics of platelet utilization pre and post transfusion guideline implementation

	Pre-Guidelines	Post-Guidelines
No. of PLT Transfusions	308	391
Apheresis/Buffy Coat PLT	129/179	198/193
No. of Recipients	48	66
Diagnosis:		
Hematologic Malignancy	18	24
Other Oncology	10	9
PICU/NICU	14	26
OR	5	4
Other	1	3
Number (%) of transfusions given with indicated pre-transfusion platelet count		
<10 x 10 <sup>9</sup> /L	45 (14.6)	36 (9.2)
<20 x 10 <sup>9</sup> /L	123 (39.9)	140 (35.8)
<50 x 10 <sup>9</sup> /L	245 (79.5)	280 (71.6)
<100 x 10 <sup>9</sup> /L	292 (94.8)	357 (91.3)

Poster # 556

## FACTORS ASSOCIATED WITH HYDROXYUREA MEDICATION ADHERENCE IN CHILDREN WITH SICKLE CELL DISEASE

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**Background:** Hydroxyurea (HDU) is recommended for older sickle cell patients with moderate to severe disease and young asymptomatic children.

**Objectives:** The objectives of the study were to understand factors related to HDU adherence, estimate the actual adherence rate among children, and to address the barriers to adherence.

**Design/Method:** Patients on hydroxyurea and their caregivers visiting our sickle cell clinic in Flint, Michigan participated in this pilot study. A questionnaire consisting of 37 questions was administered. Questions asked caregivers about their knowledge of sickle cell disease, beliefs about the effects of and the importance of giving medications, perceived barriers to regular medication taking, and demographic information. To assess actual medication-taking adherence, the caregivers received a device (GlowCap) that electronically kept track of the opening of the medication container. A specialized RN provided caregivers with education on how to use the device. The device was re-filled with a monthly supply of hydroxyurea. Because of technical issues, the length of the pilot study varied from 3 to 8 months among patients.

**Results:** Eighteen patients and their caregivers participated. Fifteen of 18 caregivers (83.3%) expressed that they had no difficulty in keeping clinic appointments. Sixteen of 18 (88.9%) stated that their children received regular checkups. Fourteen of 18 caregivers (77.8%) identified the absence of daily reminders and the absence of medication home delivery as top barriers to HDU adherence. Ten caregivers (55.6%) cited ineffectiveness of HDU as the reason for non-adherence; but, 10 caregivers also stated that HDU side effects were minimum. Using the Fisher's exact test, we detected a significant association of lower annual income with difficulty in re-filling HDU ( $p=0.046$ ). Adherence data were available for 12 participants. The mean and median adherence percentages were 76.08% (SD=20.56) and 80.50% respectively.

**Conclusion:** Barriers to HDU adherence existed. Nonadherence to hydroxyurea was not likely due to fear of side effects, and is more likely due to perception of no benefits. In collaboration with a specialty pharmacy, we are addressing these barriers by educating caregivers as to its benefits and side effects, by providing automatic HDU delivery, and by giving reminders to each caregiver to help address these barriers.



*Funded in part by Diplomat Pharmacy.*

Poster # 557

## **PHARMACOKINETIC AND COST CONSIDERATIONS FOR TRANSITION TO EXTENDED HALF-LIFE (EHL) FACTOR VIII AND IX PRODUCTS**

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**Background:** Extended half-life (EHL) factor VIII and factor IX products signal potentially important advances in the treatment of hemophilia. Although lumped into the same “extended-half life” designation, these products differ markedly in pharmacokinetic profile. The potential impact for patients with Hemophilia A and Hemophilia B must be considered separately.

**Objectives:** To investigate and compare the pharmacokinetic parameters (target peak and trough) driving prophylactic dosing regimens and the regimen-specific annual costs for standard and EHL FVIII and FIX products.

**Design/Method:** Comparative analysis of factor product coverage (standard and EHL) modeled on different half-lives and prophylaxis dosing regimens and corresponding cost analysis was performed.

**Results:** Patients with a half-life on standard Factor 8 replacement of 12 hours should achieve adequate coverage with an EHL prophylaxis regimen of 25 IU per kilogram every 3-4 days; however, those with a standard product half-life of less than 8 hours (as do many pediatric patients) will not have sufficient half-life extension to increase the dosing interval, for example, from every 48 hours to every 72 hours. Alprolix<sup>TM</sup> dosing strategy using 100 IU per kilogram every 10 days results in a 40% higher annual cost, compared to 50 IU weekly dosing, without expectation of either improved efficacy or higher trough levels. Another way of evaluating the mathematical and treatment assumptions of the models is to calculate the expected “cost per dose saved” (i.e. the annual increment in cost divided by the annual number of doses saved). For a 70 kg patient, this is an annual cost of \$209,475 per 15 doses saved (\$13,965 per infusion not given). Inadequate nominal vial size availability, particularly for pediatric patients, hinders the provider’s ability to titrate dosing.

**Conclusion:** The approved Fc-fusion FVIII and FIX products each propose two possible dosing strategies in their package insert; these strategies are *not equivalent* with respect to total factor dosage used, factor coverage, number of infusions, or cost. As stewards of the precious resource of expensive, innovative factor products, providers are obliged to think critically about the balance of convenience and cost and have frank discussions with patients about these elements before transitioning to an EHL product.

Poster # 558

## **OPTIMIZATION OF PAIN MANAGEMENT STRATEGIES IN CHILDREN WITH SICKLE CELL DISEASE AND VASO-OCCLUSIVE CRISES**

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**Background:** Vaso-occlusive crises (VOC) are a leading cause of hospital admission and healthcare cost among pediatric patients with sickle cell disease (SCD). The lack of consistent physician education regarding pain recognition and management techniques among SCD patients often results in delayed and/or inadequate analgesic administration, both extending hospitalizations and causing overall patient dissatisfaction with and distrust of the medical team.

**Objectives:** The primary objective of this study was to improve physician education and engender confidence in the care rendered, among patients admitted for VOC. Secondary objectives were to examine physician attitudes regarding VOC, and to improve the quality of pain management and overall hospital experience of SCD patients admitted to our institution with VOC.

**Design/Method:** A pediatric resident-directed educational module focusing on SCD pain and VOC management, including pain recognition strategies, appropriate analgesic dosing and alternative pain management modalities, was incorporated into resident training. A standardized admission order set for SCD VOC was implemented for use in admitting these patients to the hospital. Additionally, standardized narcotic management guidelines were provided. Resident knowledge, attitudes and comfort level regarding pain management of SCD patients was evaluated via questionnaire both prior to and following the implementation of these changes.

**Results:** Fifty-six pre-intervention surveys and 39 post-intervention surveys were completed by pediatric residents at our institution over an 18 month period. Thirty-four percent of residents felt they had been adequately trained in pain management of VOC; this number increased to 74% post-intervention. Seventy-two percent felt that VOC should last <5 days; respondents with this view decreased to 49% with education. Sixty-five percent agreed that their clinical management of VOC changed over the interventional period. Despite this, 56% of residents associated prolonged hospital stays with malingering/drug seeking and 74% felt that narcotic addiction is a concern among SCD patients. These percentages did not decrease with educational intervention.

**Conclusion:** Educational modules, standardized admission orders and narcotic management guidelines improved physician awareness and comfort levels with VOC management. However, physician misconceptions about malingering, drug seeking and addiction among SCD patients persisted, highlighting a need for further education. Future studies will examine changes in patient perspective of VOC management during this interventional period.

Poster # 559

## TESTING THE KNOWLEDGE OF HEMOPHILIA A AND INHIBITORS IN HEALTH CARE PROVIDERS

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**Background:** Hemophilia A is an X-linked disorder caused by a deficiency of factor VIII, affecting 1 in 5000 live male births. Approximately one-third of cases occur spontaneously. Treatment of hemophilia A mainly consists of FVIII replacement. Approximately 25% of patients with severe hemophilia A develop inhibitors, alloantibodies that inactivate factor VIII activity. Treatment of high-titer inhibitors requires bypassing agents (FEIBA, rFVIIa)

**Objectives:** To assess the basic hemophilia A and inhibitor knowledge of medical students and pediatric and family practice residents. We hypothesized that trainees would get <50% of the questions correct prior to dedicated education on the topic

**Design/Method:** Fifty three pediatric and family practice residents and 3rd and 4th year medical students were given a 5 question true/false survey as follows (each correct answer is 'false' except question number 3 which is a true statement): See Figure 1

This survey was followed by a 45 minute educational lecture on the topic

**Results:** Only 35% of questions were answered correctly overall. No surveys were returned 100% correct and only 3 surveys had 3 or more correct answers. See Figure 1

**Conclusion:** There is a general lack of knowledge regarding Hemophilia A and inhibitors among medical students and pediatric/family practice residents. This is possibly related to lack of formal education on the topic, which may be especially important in facilities with Hemophilia Treatment Centers where hemophilia patients are more likely to present for emergency and inpatient care.

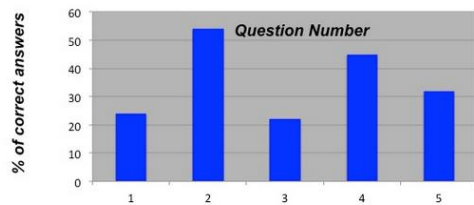


Fig 1: Percentage of correct answers by question number

**Questions:**

1. In severe Hemophilia A, FVIII:C is between 1-5%
2. 55% of patients with severe Hemophilia A develop inhibitors
3. Inhibitor is an antibody that stops clotting factor from being able to stop bleeding
4. rFVIII can effectively treat the bleeding in patients with severe Hemophilia A with high titer inhibitors
5. About one-fifth of patients who are diagnosed with hemophilia have a new mutation with no family history of hemophilia

Poster # 560

## RETROSPECTIVE STUDY OF EFFECTIVENESS OF SEPSIS PROTOCOL FOR EARLY INITIATION OF TREATMENT FOR PATIENTS WITH SICKLE CELL DISEASE

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**Background:** Fever in a patient with sickle cell disease (SCD) can suggest a life threatening etiology and is a common reason for presentation to a pediatric emergency department (ED). Time metrics surrounding treatment have been proposed as ED quality measures.

**Objectives:** To assess the effectiveness of a sepsis protocol in the early initiation of interventions in SCD patients with fever.

**Design/Method:** Retrospective review of charts selected by diagnosis of SCD by ICD-9 code. Intervention studied was a sepsis protocol developed by DCMC ED to streamline initiation of time critical interventions in high risk patients, including febrile SCD patients. Pre-intervention data included visits occurring between December 1, 2010 to April 30, 2011, while post-intervention data included visits from December 1, 2011 to November 23, 2014.

**Results:** Pre- and post-intervention, respectively, 14 and 82 patients with SCD presented with a fever (see Table 1).

**Conclusion:** After implementing a standard sepsis protocol for high risk patients, sickle cell

patients presenting with fever saw physicians more promptly, had a shorter ED length of stay, and shorter time to critical interventions, including blood culture, IVF and antibiotics. However, there was no reduction in admission rate or repeat ED visit rate. Reducing variability in the approach to febrile patients with SCD can lead to more prompt care, a reduction in time spent in the ED with no increase in revisit rate to the ED. Limitations include retrospective study design, lack of accurate time documentation and a smaller sample size in the pre-intervention group.

**Table 1:** Comparison of interventions and outcomes pre- and post-initiation of the sepsis protocol among patients with SCD who presented to ED with fever.

	Pre-intervention (n=14)	Post-intervention (n=82)
Number of repeat ED visit within 7 days	1 (7.1%)	8 (9.8%)
Number of admissions	6 (42.9%)	46 (56.1%)
Median duration of ED stay in minutes (25%-75% IQR)	227 (183 to 302)	208 (149 to 259)
Median time to intervention in minutes (25%-75% IQR)		
Time to see first physician or advanced practice provider	36 (28 to 39)	25 (18 to 34)
Time to blood culture	82 (67 to 96)	70 (40 to 105)
Time to antibiotics	100 (88 to 157)	84 (65 to 116)
Time to intravenous fluids (IVF)	105 (86 to 122)	75 (60 to 102)
Rate of intervention ordered		
Complete blood count	92.9%	97.6%
Reticulocyte count	78.6%	80.5%
Blood culture	85.7%	91.5%
IVF	64.3%	58.5%
Parental antibiotic	85.7%	82.9%
Rate of positive blood culture	0%	1.3%

Poster # 561

## HEMOPHILIA HEARTS: CARDIOVASCULAR RISK ASSESSMENT IN CHILDREN WITH HEMOPHILIA

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**Background:** An emerging health challenge in the aging hemophilia population is cardiovascular disease (CVD). CVD poses more risk to the hemophilia population than the general population since medications used to treat CVD increase the risk of bleeding, and patients with hemophilia are at substantial risk for bleeding with surgery. We hypothesized that children followed in our Hemophilia Treatment Center (HTC) have modifiable risk factors for CVD.

**Objectives:** To identify the prevalence of cardiovascular risk factors in our pediatric hemophilia population.

**Design/Method:** Retrospective chart review of males with congenital hemophilia A and B, ages 5-20-99 years, followed at Rady Children's HTC. We abstracted demographic information including race/ethnicity from the electronic health record. Body mass index (BMI) and blood pressure data were abstracted from the most recent HTC comprehensive care clinic. Descriptive statistics were used to analyze the data. Student's t-test was used to compare mean BMI % of Hispanic and non-Hispanic males in the study cohort to mean BMI % of 5-19 y old white

Hispanic and non-Hispanic males included in NHANES 2011-2012.

**Results:** Seventy-four males were included, 60% Hispanic. The mean age is  $12.4 \pm 4.3$  years. The mean BMI % is  $70.8 \pm 27$  with 5.4% underweight, 54% healthy weight, 17.6% overweight and 23% obese. Blood pressure readings were available for 73 children; 13.7% have blood pressure consistent with prehypertension and 21.9% consistent with hypertension. Sixty-five White males were included in the NHANES comparison. The males in our cohort had a higher mean BMI compared to males in NHANES (72.8% v. 65.7%;  $p=0.046$ ). Hispanic males in our cohort had a higher BMI compared to Hispanic males in NHANES (78.8% v. 70.1%,  $p=0.025$ ). There was no difference in BMI between the non-Hispanic Whites.

**Conclusion:** We identified a significant proportion of children with hemophilia with modifiable risk factors for CVD. Overweight/obesity is particularly high in our Hispanic population. The next step is a cross-sectional study of family history, nutrition, physical activity, and health literacy. Our goal is to develop a multi-disciplinary approach to identify modifiable risk factors for CVD and to promote cardiovascular health and risk reduction during HTC comprehensive care clinics.

Poster # 562

## **THE USE OF MOBILE TECHNOLOGY TO PROVIDE INTENSIVE TRAINING FOR ORAL CHELATION MANAGEMENT IN PEDIATRIC SICKLE CELL DISEASE AND BETA-THALASSEMIA**

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**Background:** Pediatric patients with  $\beta$ -thalassemia require lifelong transfusions, and patients with sickle cell disease (SCD) increasingly receive chronic transfusions to prevent and treat complications. However, these patients are at risk of iron overload and associated complications, including organ failure and death. To prevent these risks and optimize health outcomes, adherence to daily oral chelation therapy is required.

**Objectives:** We developed a mobile health application to assist in an Intensive Training Program (ITP) for chelation management, consisting of medication tracking through video “selfies” and logs, education modules, patient-provider communication and long-term follow-up. We aimed to determine compliance, identify changes in ferritin, and facilitate disease knowledge.

**Design/Method:** Pediatric patients receiving chronic RBC transfusions and chelation therapy were eligible. Patients participated in provider-led education modules, completed disease knowledge assessments, and utilized our mobile application to track daily medication adherence via “selfies” and, after intensive training (days 0-90), via self-report log.

**Results:** Twelve patients participated (mean age=12.9 years; SCD=10;  $\beta$ -thalassemia=2). Baseline compliance data indicated 45.4% patients missed 0-1 weekly doses, 27.3% missed 2-3 weekly doses, and 27.3% missed 4 or more weekly doses. We analyzed 1627 data days for 11 patients (one patient excluded given technical difficulties) who were followed for an average of 158.9 participation days (range 115-185).

On average, patients tracked their medication utilizing “selfies” 81% of days the first 30-days; reported 80% compliance at 90-days; and self-reported 85% compliance following ITP completion. Paired t-test analyses indicated that ferritin levels trended toward significant improvement following conclusion of the 90-day ITP when compared to baseline. At 4-month follow-up, mean ferritin levels decreased by 237.55 ng/mL ( $t=1.2$ ,  $p=0.12$ ), and at 5-months,

mean ferritin levels decreased by 591.3 ng/mL ( $t=1.5$ ,  $p=0.09$ ). Disease knowledge assessments revealed a high level of retention after 90-days (96%).

**Conclusion:** Following ITP completion, we found high levels of medication compliance (>80%) via application tracking of daily adherence. Additionally, while ferritin values demonstrated only a trend toward statistical significance, mean levels were clinically improved. Thus, we are optimistic towards the use of our mobile app and ITP to facilitate compliance, disease knowledge, and improved health outcomes for these children.

Poster # 563

## **SAFETY AND EFFICACY OF LONG-TERM OPEN-LABEL DOSING OF ROMIPLOSTIM, A THROMBOPOIETIN (TPO)–RECEPTOR AGONIST, IN THROMBOCYTOPENIC PEDIATRIC PATIENTS WITH IMMUNE THROMBOCYTOPENIA (ITP) IN AN EXTENSION STUDY**

James Bussel, Michael Tarantino, Amy Geddis, Michael Guerrero, Alan Ikeda, Kun Nie, Janet Franklin, Melissa Eisen

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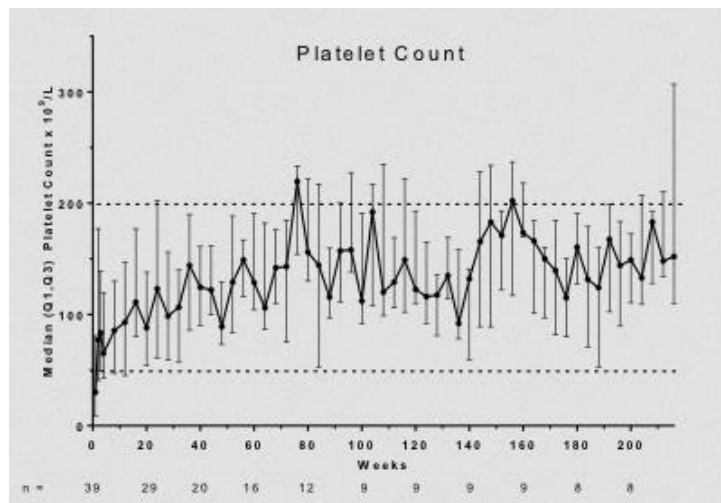
**Background:** Children with chronic ITP from a phase 1/2 study and an ongoing phase 3 study entered an open-label extension study of romiplostim.

**Objectives:** Evaluate long-term romiplostim in pediatric ITP.

**Design/Method:** Weekly subcutaneous romiplostim (up to 10 µg/kg) targeted platelet counts of  $50\text{--}200 \times 10^9/\text{L}$ .

**Results:** Forty patients entered the extension (12 from phase 1/2; 28 from phase 3); 39 received romiplostim  $\leq 217$  weeks (4.2 years). Median (range) treatment duration was 40 (5–217) weeks; number of doses 37 (5–216); average weekly dose 6 (0–10) µg/kg; maximum dose was 8 (1–10) µg/kg. Ten patients discontinued, none because of adverse events (AEs). Median platelet counts were  $\geq 50 \times 10^9/\text{L}$  after week 1 (**Figure**). Two patients achieved remission (platelets  $> 50 \times 10^9/\text{L}$  for  $\geq 24$  weeks without ITP treatment); one patient started romiplostim after having ITP for 2 years, the second after 11 months. Median doses remained  $\leq 6$  µg/kg. Eleven patients (21 instances) received rescue medications (for platelets  $< 10 \times 10^9/\text{L}$ , bleeding/wet purpura, or investigator decision) mostly in the first 3 months (12 instances). Seven patients had 13 serious AEs (asthma, epistaxis, gastroenteritis, gastrointestinal infection, hemangioma, infection, mouth hemorrhage, pain, pharyngitis, pyrexia, tachycardia, transfusion reaction, and viral infection); 3 had life-threatening AEs (infection,  $n=1$ ; thrombocytopenia,  $n=2$ ), none fatal or deemed treatment-related. Thirty patients had bleeding AEs, including contusion ( $n=11$ ), epistaxis (9), petechiae (8), and gingival bleeding (7).

**Conclusion:** Long-term treatment with up to 4.2 years of romiplostim maintained platelet counts in children with chronic ITP without significant toxicity. Tachyphylaxis was not seen and rescue medication use diminished with time.



Poster # 564

## REALIZING EFFECTIVENESS ACROSS CONTINENTS WITH HYDROXYUREA: THE REACH TRIAL

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**Background:** Sickle cell anemia (SCA) is an important global health problem, contributing substantially to under-5 mortality, particularly in sub-Saharan Africa. Several African countries are developing national SCA strategies that focus on early diagnosis and prophylaxis with penicillin and pneumococcal immunization. Introducing disease-modifying therapy with hydroxyurea now is a logical next step since other treatment options (transfusions, transplantation) are typically not safe, affordable, or readily available in Africa. Although hydroxyurea has proven efficacy in the US, comorbidities of African children with SCA may affect safe dosing and reveal more significant toxicities.

**Objective:** REACH aims to obtain pilot data regarding the safety, feasibility, and benefits of hydroxyurea for children with SCA in sub-Saharan Africa. The long-term goal is to generate data so that hydroxyurea can be included in national SCA strategies.

**Design/Method:** REACH (NCT01966731) is a prospective, phase I/II open-label dose escalation trial of hydroxyurea that will enroll and treat 600 children, age 1-10 years, at 4 clinical sites in sub-Saharan Africa (Democratic Republic of Congo, Kenya, Angola, and Uganda). After a six-month fixed-dose phase at 15-20 mg/kg/day and six-month dose escalation phase to maximum tolerated dose, hydroxyurea will continue for 3 more years.

**Results:** REACH is approved by Ethics Boards at three clinical sites; enrollment commenced July 2014 in the Democratic Republic of Congo and September 2014 in Kenya. To date, 115 participants have enrolled with only 4 screening failures; 75 children have initiated hydroxyurea therapy. Baseline growth parameters reveal an average weight-for-height Z-score of -0.76 and -0.91 at the two enrolling sites, and average height-for-age Z-score of -0.91 and -1.18, respectively. Baseline laboratory parameters document an average hemoglobin concentration of 7.0 and 7.7 gm/dL, and absolute reticulocyte counts of 383 and 313 x 10<sup>9</sup>/L, respectively.

**Conclusion:** REACH is the first multi-center study of hydroxyurea in Africa and was written in

full partnership with local clinical investigators. Enrollment has been brisk at two clinical sites, with plans to activate two additional sites in 2015. Baseline parameters document substantial growth delay and severe hemolytic anemia, which will allow REACH to assess the feasibility of hydroxyurea treatment in the setting of substantial comorbidities.

Poster # 565

### **A Pilot Study to Assess Health-Related Quality of Life (HRQoL) in Older Children and Adolescents with Primary Immune Thrombocytopenia (ITP)**

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**Background:** ITP is an immune-mediated disorder of platelet destruction and platelet production that may result in chronic, symptomatic thrombocytopenia. The impact of chronic ITP (cITP) on HRQoL in children is not well defined.

**Objectives/Design/Method:** We administered a validated, age-appropriate HRQoL instrument, the Kids ITP Tools (KIT) (Klaassen, 2007) consisting of 27 questions to assess the HRQoL of older children and adolescents with cITP, and the consequential burden on their caregivers. Numerical KIT scores, are directly related to HRQoL. PDSA invited participants (8-18 years old) and their parents to complete an online KIT survey and a demographic and clinical questionnaire.

**Results:** Data were available for 70 children, 155 parents, and 80 parents-as-proxies for their children. Pediatric participants had a male-to-female ratio of 0.98, and a mean age/duration of ITP of 12.8/5.6 years, respectively. Bleeding was reported in 97% and drug treatment for ITP in 94%; 44% within the last month. Team sports or club participation was reported in 42.9% and 54.3%, respectively. Twenty percent reported having been bullied. KIT scores were not significantly different by age, recent treatment or bleeding symptoms. The younger cohort (8-12 years), reported a worse HRQoL than the older cohort, 12-18 years ( $p=0.042$ ). A worse HRQoL was reported by children with ITP < 3 years versus ITP  $\geq$  3 years ( $p=0.031$ ), > 5 treatment AEs versus  $\leq$  5 treatment AEs ( $p=0.010$ ), no sports participation versus sports participation ( $p=0.009$ ). Parents-as-proxies reported a worse HRQoL for children with ITP for < 3 years ( $p=0.008$ ), > 5 treatment AEs ( $p=0.003$ ), co-morbidities\* ( $p=0.009$ ), and no sports participation ( $p=0.002$ ). Parental burden was reported as worse if their affected child was younger ( $p=0.025$ ), non-Caucasian ( $p=0.025$ ), had ITP < 3 years ( $p=0.026$ ), had co-morbidities\* ( $p=0.048$ ), or refrained from social clubs ( $p=0.046$ ). Child and parent-as-proxy responses were highly correlated,  $r^2 = 0.8$ . \*allergies; anemia; diabetes, disorders of the ears, heart, intestines, kidney, lungs, stomach, skin, thyroid; genetic syndromes, recurrent infections, seizures

**Conclusion:** This pilot study illuminates the somewhat unpredictable determinants of HRQoL in children with cITP and the burden on their parents and will facilitate larger cross-sectional HRQoL study of children and adolescents with cITP.

Poster # 566

### **TARGETING MICROPARTICLE PRIMING OF NEUTROPHILS DURING SICKLE CELL VASO-OCCLUSIVE PAIN CRISES**

Christopher McKinney, Marguerite Kelher, Rachelle Nuss, Christopher Silliman



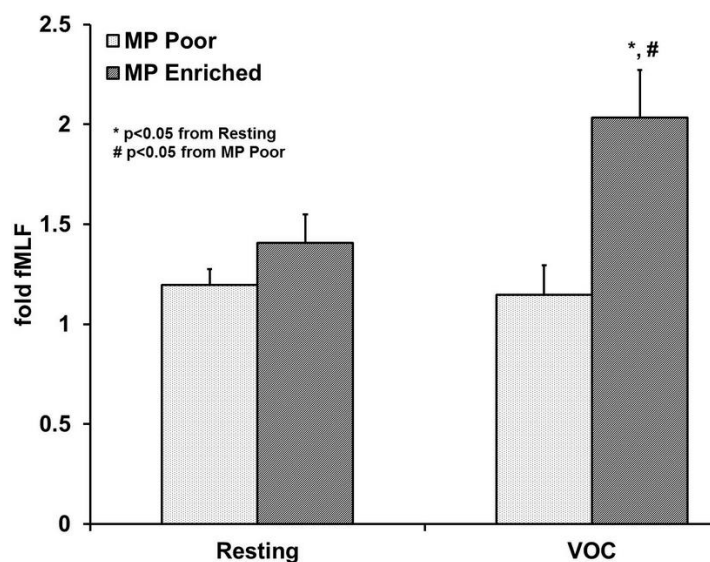
**Background:** Microparticles (MPs) are submicron exosomes released by cells undergoing activation or apoptosis and have been implicated in a variety of inflammatory disorders. Circulating MPs are increased in patients with sickle cell disease (SCD) and increase further during painful crises.

**Objectives:** To determine the role of MPs in the inflammatory pathophysiology of SCD by studying their effect on neutrophil (PMN) priming and endothelial cell activation and to evaluate possible inhibition of these mechanisms by simvastatin.

**Design/Method:** Blood was obtained from patients with sickle cell disease during steady state and episodes of vaso-occlusive pain crises (VOC) or acute chest syndrome. MPs were isolated via differential centrifugation and measured by flow cytometry. To evaluate priming, neutrophils were isolated from healthy donors and then incubated with the isolated MPs in the presence or absence of 1  $\mu$ M simvastatin for 10 minutes. PMN priming was measured as the augmentation of the fMLF-activated respiratory burst. Human microvascular pulmonary endothelial cells were incubated with 1  $\mu$ M simvastatin for 1 hour and with MPs for 6 hours. Surface CD54 expression was measured via flow cytometry.

**Results:** PMN priming by MPs is increased during VOC (Figure 1). Preliminary data suggest that simvastatin inhibits PMN priming by 14% and endothelial cell activation by 19.6%.

**Conclusion:** Circulating MPs in patients with SCD may contribute to inflammation by increasing PMN priming activity. Simvastatin can inhibit PMN priming and endothelial cell activation. This inhibition provides additional pre-clinical evidence for the potential utility of statin therapy in SCD patients.



Poster # 567

## THROMBOELASTOGRAPHY TESTING IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA TREATED WITH PEG-ASPARAGINASE

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**Background:** Children with acute lymphoblastic leukemia (ALL) receiving asparaginase during induction chemotherapy are at risk of developing thrombotic and bleeding complications. Thromboelastography (TEG) is a global assay that simultaneously measures multiple components of the hemostatic system. TEG is used to monitor hemostasis and guide transfusion practices in conditions that result in complex disorders of hemostasis including trauma and liver disease. **Objectives:** To characterize the hemostatic abnormalities induced following the administration of PEG-Asparaginase to children with ALL using thromboelastography **Design/Method:** TEG was evaluated in 23 newly diagnosed pediatric patients with ALL getting a single dose of PEG-Asparaginase during induction therapy. Coagulation profile, antithrombin III & thromboelastography tests were done prior and on 4 days (+/- 2 days) and 18 days (+/- 2 days) after the administration of PEG-Asparaginase. **Results:** There was a significant decrease in angle(A), clot strength(MA) and fibrinolysis(LY30) measured by TEG following PEG-Asparaginase, which indicate an increased risk for bleeding. There was a significant decrease in antithrombin III suggesting increased risk of thrombosis. There was no significant change in clotting time(R) on TEG. Results are summarized in Table 1. **Conclusion:** TEG is a comprehensive and efficient test that can detect and characterize hemostatic changes caused by PEG-Asparaginase in ALL patients. TEG monitoring after asparaginase therapy may provide valuable information about the dynamic changes that occur in hemostasis and has the potential to identify high risk patients that may benefit from intervention. Further studies are necessary to understand the extent of hemostatic changes and to standardize these assays.

Table 1. Results of TEG and coagulation studies						
N= 23		Mean +/- Standard Deviation				
Variable	Normal values	Measurement	Base line	2-6 days post	16-20 days post	Overall P-value
TEG parameter						
R	5 - 10 min	Clotting Time	6 ± 1.4	6.7 ± 2.8	5.6 ± 1.3	0.14
A	50 - 70 degree	Clot Kinetics	56.8 ± 15.9	43.6 ± 18.9	51.7 ± 12.3	<0.01*
K	1 - 3 min		2.8 ± 2.1	5.4 ± 4.9	5 ± 4.5	0.1
MA	50 - 70 min	Clot Strength	47.1 ± 14.8	36.8 ± 12.8	39.7 ± 11	<0.01*
Ly30	0 - 8%	Clot Stability	0.6 ± 0.9	0.8 ± 2.5	4.5 ± 9.4	0.04*
Coagulation parameters						
Platelets	150 - 400 K		67.7 ± 53.3	56.2 ± 47.1	128 ± 69.5	<0.01*
PT	13 - 15 sec		15.4 ± 1.5	15.6 ± 3.9	16.3 ± 2.1	<0.01*
PTT	24.5 - 37.5 sec		29.8 ± 4.7	33.6 ± 6.2	32.4 ± 5.7	0.019*
AT III	79 - 131 %		120 ± 19.7	79.9 ± 15.7	64.1 ± 20.1	<0.01
Fibrinogen	164 - 382 mg/dl		278.4 ± 124.4	123.7 ± 42.7	119.8 ± 101.1	<0.01
N= Number of patients R= Enzymatic reaction, indicating clotting factors A= Rapidity of fibrin build up and clot strengthening, representing fibrinogen level K= Speed to reach amplitude of 20 mm MA= Function of platelet and fibrinogen bonding LY30= Amplitude reduction 30 min after MA, indicating fibrinolysis						

Poster # 568

## DIFFUSE MYOCARDIAL FIBROSIS IS A NOVEL MECHANISM OF CARDIOMYOPATHY IN SICKLE CELL DISEASE

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**Background:** Cardiopulmonary disease is the leading cause of mortality in sickle cell disease (SCD). Restrictive physiology, defined by diastolic dysfunction, left atrial dilation and normal systolic function is common in SCD, but the cause is poorly understood. Diffuse myocardial fibrosis is a common cause of restrictive cardiac physiology. Focal or macroscopic scarring and fibrosis are rare findings in SCD, whether on biopsy or by late gadolinium enhancement (LGE) using cardiac magnetic resonance (CMR) imaging. Diffuse myocardial fibrosis is not detected by LGE but can be quantified using a novel CMR T1 mapping technique to measure extracellular volume (ECV), which correlates with histologic microfibrosis.

**Objective:** Assess for diffuse myocardial fibrosis using CMR and ECV measurements in SCD.

**Design/Method:** Children and adults with SCD (HbSS) prospectively underwent CMR to evaluate heart chamber size and performance. ECV was measured from T1 maps using a modified Look-Locker inversion recovery (MOLLI) sequence obtained pre- and post-gadolinium injection, by the following formula: myocardial ECV =  $(1 - \text{hematocrit}) \times (\Delta R1_{\text{myocardium}} / \Delta R1_{\text{blood}})$ , where R is relaxation time. M-mode echocardiography and tissue Doppler imaging were used to measure early mitral inflow (E) and mitral annular velocities (e').

**Results:** We studied 19 SCD patients, median age 19 years (range 6-48). No patient had focal scarring or delayed enhancement (LGE). Seventeen of 19 (89%) patients had elevated ECV. ECV was significantly higher in SCD patients compared to normal (mean  $35 \pm 5\%$  vs  $25 \pm 2\%$ ,  $P < 0.001$ ). ECV was associated with hemoglobin ( $R = -0.46$ ,  $P = 0.048$ ) and reticulocytes ( $R = 0.61$ ,  $P = 0.005$ ). Restrictive physiology was observed in many: 6/17 (35%) had severe diastolic dysfunction defined by severely abnormal lateral and septal E/e' ratios; 10/19 (53%) patients had significantly enlarged left atrial volume (LAV) (mean LAV index  $53.5 \pm 11.7 \text{ ml/m}^2$  [normal  $< 40 \text{ ml/m}^2$ ]); and all had normal systolic function. Patients with increased LAV had significantly higher ECV ( $38 \pm 5\%$  vs  $33 \pm 4\%$ ,  $P = 0.02$ ).

**Conclusion:** Elevated ECV is a common abnormality in SCD, suggesting presence of diffuse myocardial fibrosis. Myocardial fibrosis, diastolic dysfunction, and LA dilation are often concordant, and degree of LA dilation correlates with fibrosis, consistent with restrictive physiology. Diffuse myocardial fibrosis is a potential mechanism of cardiac pathology in SCD that may result in a unique SCD-related cardiomyopathy with restrictive physiology.

Poster # 569

## Hematologic Characteristics and Management of Neonatal Stroke

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**Background:** Few large scale studies have been conducted on neonatal stroke. Additionally, these studies have focused on the single characteristics of stroke and there have been variable results across all studies. There are a few hypothesized risk factors for neonatal stroke however according to the International Paediatric Stroke Study 47% of ischemic strokes have no risk factors. Current standard of care for evaluation of a neonate with stroke includes a minimum of activated protein C (APC) and/or Factor V Leiden (FVL), prothrombin gene mutation, and protein C & S deficiency.

**Objectives:** Our objective was to determine if current Southern California Permanente Medical Group (SCPMG) NICU practice was meeting standards of care by identifying neonates who incurred stroke and who had a hypercoagulable workup completed.

**Design/Method:** Southern California Permanente Medical Group (SCPMG) NICU retrospective

chart review of infants who incurred stroke in the first 28 days of life between 01/01/2004-12/30/2014 was completed. All infants with both ischemic and hemorrhagic stroke were included. Stroke had to be confirmed on either brain MRI or CT.

**Results:** A total of 84 patients were identified. Of these 84, 40 had both a protein C & S checked, 35 had a anti thrombin gene mutation checked, 27 had a homocysteine checked, 22 had both anticardiolipin and lupus anticoagulant checked, 20 had a FVL checked, 19 had an APC checked, 15 had a prothrombin gene checked, and 7 had a beta 2 glycoprotein checked. Additionally, there was inconsistency in which hypercoaguable labs were obtained for each patient, with no standardization.

**Conclusion:** Given the variability in the hypercoaguable work up, we propose completing the currently recommended lab tests with the addition of anticardiolipin antibody, lupus anticoagulant, and beta 2 glycoprotein. Given SCPMG's heterogenous patient population these results could help identify those genetically predisposed for recurrent stroke. However, further review of those with positive tests is needed to determine whether an expanded standardized lab panel is necessary to identify those at risk for recurrent stroke.

Poster # 570

## **PRIMARY CARE-SPECIALTY PARTNERSHIPS TO IMPROVE OUTCOMES IN SICKLE CELL TRANSITION CARE**

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**Background:** The transition between pediatric and adult care is a vulnerable period for sickle cell patients. [i] Historically, University of Rochester Division of Pediatric Hematology transitioned patients to adult hematology based on age or need in a non-standardized fashion. Subsequently, most crises were managed inpatient or in the emergency department (ED). In fiscal year 2012, this resulted in 534 admissions or ED visits for patients aged 19 and older, amounting to \$218,000. We identified an internal medicine-pediatrics (med-peds) practice to care for patients post transition, including pain management, which we would expect to reduce acute care utilization.

**Objectives:** To design a sickle cell transition program which reduces acute care visits with support from a primary care program.

**Design/Method:** We developed a collaborative transition program between pediatric hematology and a primary care med-peds practice. Seventy-seven patients were identified between ages 14 and 21 who would be offered transition readiness assessments based on the HRSA funded 'Got Transition' toolkit [ii] during clinic visits. A personalized curriculum based on published sources [iii] and knowledge gaps will be developed for each patient. A quarterly meeting comprising pediatric and adult hematology, med-peds, social work, and administrators reviews upcoming transitions. Patients will then transition to adult primary care, preferentially to the med-peds practice. Once primary care is established, patients will be referred to adult hematology. We will examine clinical outcomes including patient readiness, ED and inpatient utilization, and patient engagement in the med-peds practice before and after transition.

**Results:** Prior to our intervention, no patients had been transitioned in the preceding 12 months. In this first year of the program, transition has been initiated for 30 eligible patients. Nine of these 30 have undergone formal transition assessment.

**Conclusion:** We demonstrated the creation of a multidisciplinary team-based model of care for

sickle cell patients. Early results from transition assessments suggest patients respond favorably to a formal approach. We continue to gather data on costs and utilization of care. Collaboration with local med-peds primary care groups provides an opportunity to improve transition outcomes for sickle cell patients.

[i] Quinn, Blood, 2010.

[ii] [www.gotttransition.org](http://www.gotttransition.org) (accessed 1/5/15)

[iii] Hankins, WISCH, 2013.

Poster # 571

## **EVALUATION OF THE SAFETY AND EFFICACY OF A HOME MONITORING PROGRAM FOR CHILDREN RECEIVING CHRONIC WARFARIN THERAPY**

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**Background:** Point-of-care (POC) international normalized ratio (INR) testing is used widely among patients receiving warfarin therapy. However limited data are available on the safety and effectiveness of POC testing in children and young adults, and whether families can be taught to self-dose-adjust under practitioner guidance.

**Objectives:** We evaluated the safety and efficacy of POC testing and self-dose adjustments in children and young adults.

**Design/Method:** Part I consisted of utilizing the CoaguChek XS® at home with INR adjustments made by the practitioner. Part II consisted of self-dose adjustments based on home INR results with practitioner guidance. Three cohorts were chosen: (1) new to warfarin, (2) established on warfarin, and (3) low-adherence. An education program was completed prior to entry into both parts of the study. The primary endpoint was percent of time in the therapeutic range (TTR) and incidence of thrombotic and bleeding adverse events.

**Results:** Of 124 eligible patients, 30 were enrolled. Median age was 16.4 years (range 4 -25). Average TTRs for patients in cohorts 1 and 2 were 60% (SD 27%) and 73% (SD 18%), respectively (p=0.24). Cohort 2: INR control was no different before and after transition to a home INR monitor (TTR 69% [lab] vs. 73% [home], P=0.74). Cohort 1: adherence was 100% during home testing. Cohort 2 patients: adherence with INR tests was 98% during lab testing and 100% during in-home testing. Cohort 3 failed to enroll. Of the 30 enrolled patients, 12 were eligible for part II of whom 8 consented. During the three-month self-dose adjustment period, the TTRs of the 8 patients were not significantly different (76% for part I vs. 70% for part II, P=0.36). None of the 15 self-dose titrations were deemed to be incorrect or unsafe by the practitioners. There were no bleeding or thromboembolic events in either part of the study.

**Conclusion:** POC testing and self-dose adjusting can be performed with safety and effectiveness in adherent families. Adherence with INR testing was at least as good as in-laboratory testing. Low-adherence patients could benefit from home INR monitoring, but our data suggest they are difficult to recruit for research studies.

Poster # 572

## INFLUENCE of APOL1 POLYMORPHISMS ON ALBUMINURIA IN CHILDREN WITH SICKLE CELL ANEMIA

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**Background:** Nephropathy in sickle cell anemia (SCA) begins in childhood and portends early mortality. *APOL1* gene variants are associated with non-diabetic renal disease among African-Americans, but have not been investigated in children with SCA.

**Objectives:** Determine the frequency of *APOL1* variants among children with SCA and investigate associations with albuminuria.

**Design/Method:** DNA from 85 children (age 3-18 years) enrolling in Hydroxyurea Study of Long-Term Effects (HUSTLE, NCT00305175) was genotyped for *APOL1* single nucleotide polymorphisms (SNPs) that form the G1/G2 alleles. G1 is defined by two missense variants (rs73885319 and rs60910145) while G2 is a nucleotide deletion (rs71785313). Albuminuria ( $\geq 30$  mg protein/gm creatinine) was associated with G1/G2 alleles by correlation trend statistical tests. The entire *APOL1* gene was examined by whole exome sequencing (WES) to identify other *APOL1* variants associated with albuminuria.

**Results:** Albuminuria was present in 14 patients (16.5%). *APOL1* variants were common (G1 allele frequency = 21.8%, G2 allele = 15.9%, Table). Children with one or two G1 alleles had a significantly increased risk of albuminuria ( $p=0.018$ , Odds Ratio 3.4 [C.I. 1.0- 11.9]), but G1 was not associated with serum creatinine or glomerular filtration rate. In contrast, the G2 allele was not associated with albuminuria. WES identified 8 additional non-synonymous *APOL1* variants, but none was associated with albuminuria.

**Conclusion:** The *APOL1* G1 allele is associated with the presence of albuminuria among children with SCA, with highest prevalence in G1 homozygotes. Identification of children at higher risk for renal disease could lead to enhanced screening and earlier intervention.

Genotype	Prevalence		Albuminuria			p-value
	N	(%)	Negative	Positive	% positive	
G0/G0	30	35.3	26	4	13.3	NS
G0/G2	22	25.9	21	1	4.5	
G2/G2	1	1.2	1	0	0.0	
G0/G1	24	28.2	18	6	25.0	0.018
G1/G1	5	5.9	3	2	40.0	
G1/G2	3	3.5	2	1	33.3	

Poster # 573

## CONTINUOUS RECOMBINANT HUMAN ANTITHROMBIN INFUSION TO OVERCOME HEPARIN RESISTANCE IN NEONATES AND YOUNG INFANTS WITH LIFE-THREATENING THROMBOSIS

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**Background:** Sick neonates and infants with extensive thrombosis often exhibit relative “heparin resistance” due to accelerated heparin clearance and lower endogenous antithrombin (AT) levels, leading to suboptimal anticoagulation. AT concentrates are increasingly used off-label in critically ill neonates and children to optimize heparinization. Recombinant human AT (rh-AT) concentrate with its short half-life allows for continuous infusion administration and maintenance of consistent plasma AT levels resulting in improved heparin responsiveness and stable anticoagulation.

**Objectives:** We report two neonates and one young infant with life-threatening thrombotic events and heparin resistance successfully managed with continuous rh-AT infusion.

**Design/Method:** Case series

**Results:** Refer to summary table. Three patients (2 neonates, 1 young infant) diagnosed with extensive venous thrombosis were started on heparin anticoagulation. Due to difficulty achieving therapeutic anticoagulation despite standard dosing and appropriate escalation, low baseline AT levels, and the presence of extensive multi-segment thrombi, rh-AT infusion was started according to manufacturer’s instructions, though without the initial bolus to minimize bleeding risk. Infusion was titrated to maintain AT levels of 80-120% with close laboratory monitoring. AT and heparin-anti-Xa levels increased and were maintained within goal with relative stability in all three patients. Therapeutic heparinization was achieved  $\leq 24$  hours after starting rh-AT infusion. Importantly, rh-AT infusion was well-tolerated with no major bleeding complications and with good outcomes.

**Conclusion:** Our experience suggests that rh-AT infusion is efficacious and safe in optimizing heparinization in neonates and young infants with serious thrombosis.

**Summary Table**

Patient age at start of rh-AT infusion	Extent of thrombosis/ Other characteristics	Type of heparin while on rh-AT infusion	Baseline anti-Xa (units/mL)	Baseline AT (%)	AT on rh-AT infusion (%)	Anti-Xa on rh-AT infusion (units/mL)	Duration of rh-AT infusion (days)	Outcome
6 days	Lower venous system thrombosis (IVC, bilateral renal veins) with acute renal failure	UFH infusion, LMWH	0.05-0.23	38	65-104	0.2-0.65	13	Alive Mild renal insufficiency No major bleeding
9 days	Cerebral sinovenous thrombosis (bilateral transverse, straight sinuses) with raised ICP	UFH infusion	0.05-0.17	41	71-103	0.33-0.69	5	Alive Mild developmental delay No major bleeding
6 weeks	Upper venous system thrombosis (SVC; bilateral internal jugular, bilateral subclavian, left brachiocephalic veins; bilateral transverse sinuses) with SVC syndrome	LMWH	0.18-0.35	37	79-96	0.6-1.2	27	Alive Resolution of SVC syndrome No major bleeding
<b>Abbreviations:</b> AT = antithrombin, rh-AT = recombinant human antithrombin, UFH = unfractionated heparin, LMWH = low molecular weight heparin, IVC = inferior vena cava, SVC = superior vena cava, ICP = intracranial pressure								

Poster # 574

## EFFECTS OF GENETIC POLYMORPHISMS ON LEUKOCYTE AND NEUTROPHIL COUNTS ON CHILDREN WITH SICKLE CELL ANEMIA



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**Background:** Elevated white blood cell (WBC) count is associated with morbidity and mortality in sickle cell anemia (SCA), but allows a higher hydroxyurea maximum tolerated dose (MTD). Single nucleotide polymorphisms (SNPs), especially Duffy Antigen Receptor for Chemokine (DARC) variants, influence WBC and absolute neutrophil count (ANC) in the general population and adults with SCA, but their effects on children with SCA are unknown.

**Objectives:** Determine the effects of candidate SNPs on baseline WBC and ANC among children with SCA, identify novel variants affecting WBC and ANC, and investigate their effects on hydroxyurea MTD.

**Design/Method:** DNA (N=180) from Hydroxyurea Study of Long-Term Effects (HUSTLE, NCT00305175) was genotyped for candidate SNPs. Associations with baseline WBC, baseline ANC, and hydroxyurea MTD were tested using an additive model and correlation trend test. Whole exome sequencing (WES) data were examined for WBC and ANC associations.

**Results:** rs2814778 regulating Duffy antigen expression had a minor allele frequency (MAF) of 11.9%. Children with  $\geq 1$  Duffy allele had significantly higher WBC ( $15.7$  vs.  $13.5 \times 10^9/L$ ) and ANC ( $8.0$  vs.  $6.5 \times 10^9/L$ ) compared to Duffy null Fy(a-b-) ( $p < .005$ ). For rs12075, which distinguishes the major Duffy alleles, Fya was associated with higher ANC. Hydroxyurea MTD was associated with WBC ( $p = 0.01$ ) but not ANC or candidate SNPs. WES (N=128) identified 33,304 non-synonymous variants with allele frequency  $> 2\%$ , including 22 novel SNPs significantly associated with both WBC and ANC ( $p < .001$ ).

**Conclusion:** Common genetic variants affecting WBC and ANC in the general population also modify the phenotype of children with SCA.

Gene	SNP	Location	MAF	Baseline WBC		Baseline ANC	
				r value	p value	r value	p value
DARC	rs2814778	1q23	.119	.226	.004	.228	.004
DARC	rs12075	1q23	.033	.114	NS	.197	.013
IFI16	rs4657616	1q23	.045	.229	.004	.116	NS
PSMD3-CSF	rs4065321	17q21.1	.374	-.030	NS	-.072	NS
CDK6	rs445	7q21.2	.180	-.025	NS	-.061	NS
CXCL2	rs9131	4q13.3	.209	.000	NS	-.046	NS

Poster # 575

## **TREATMENT OUTCOMES IN PEDIATRIC PATIENTS WITH PULMONARY EMBOLISM USING LOW DOSE TISSUE PLASMINOGEN ACTIVATOR THERAPY**

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**Background:** Based on ACCP guidelines, unfractionated heparin or low-molecular-weight heparin (LMWH) remains standard therapy for Pulmonary Embolism (PE) and systemic thrombolysis is reserved for life threatening thrombosis due to concerns with bleeding.

Thrombolytic therapy has been shown to be more efficacious than anticoagulation alone in adults with PE but similar data in pediatrics is lacking. Specific guidelines for use of low dose tissue



plasminogen activator (LD-TPA) in children with PE were created at Children's Hospital of New Orleans. We have used systemic LD-TPA (0.03 mg/kg/hr initially, increased to 0.05 mg/kg/hr if less than optimal response within 24 hrs) together with unfractionated heparin (5 units/kg/hr) to achieve a goal PTT of 40-60 seconds. LD-TPA is continued until clot resolution is achieved, for a maximum of 72 hrs, when patients are transitioned to LMWH.

**Objectives:** To determine effectiveness and if there are increased bleeding complications associated with systemic LD-TPA in treatment of PE in children.

**Design/Method:** Retrospective chart review of patient incidences (PI) with PE and treated with LD-TPA between June 2008 and June 2014 was performed. Underlying risk factors for thrombophilia were noted. Responses were classified as complete resolution of thrombus (CR), improvement but no CR (PR) or no change (NR) based on imaging at the end of LD-TPA treatment. Data were analyzed to determine the efficacy of LD-TPA.

**Results:** Sixteen PI with PE were identified, for which LD-TPA was used (Median age = 13 years). Mean duration of treatment was 51 hrs (range 17-72 hrs).

Seven PI needed increase in dose of TPA to 0.05 mg/kg/hr after 24 hrs due to PR or NR.

Nine (56%) PI had CR, six (38%) were PR and one (6%) was NR.

One bleeding episode requiring discontinuation of TPA occurred in a patient with gun-shot injury in whom LD-TPA was relatively contraindicated but was started at treating physician's discretion.

Six (38%) had minor bleeding episodes [epistaxis (2), oozing from IV sites (3), positive fecal occult blood (1)].

**Conclusion:** To our knowledge, this is first clinical study of LD-TPA for PE in pediatrics. Our results show that LD-TPA is effective and relatively safe in treatment of PE in children.

Poster # 576

## **DEVELOPMENT OF QUALITY MARKERS FOR THE TRANSITION FROM PEDIATRIC TO ADULT CARE IN SICKLE CELL DISEASE FROM THE PERSPECTIVE OF ADULT PROVIDERS USING A MODIFIED DELPHI METHOD**

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**Background:** Transition from pediatric to adult care is a vulnerable time for young adults with sickle cell disease (SCD). Improving transition practices is limited by the lack of established quality indicators.

**Objectives:** Objective: To create quality metrics for transition in SCD.

**Design/Method:** We reviewed the literature and drafted a total of 23 candidate quality transition measures in three categories; transition and transfer process measures, patient factors and transition outcomes. Using a modified Delphi survey an expert panel (members of the sickle cell adult provider network (SCAPN)) rated each measure based on importance and feasibility of measurement. Because all proposed measures received an importance rating of 7-9 on a 9-point Likert scale (above the predetermined cut-off for retention), in round #2 each respondent also chose a 'top 5' list of the most important measures in each category.

**Results:** Of 190 SCAPN members, 106 (56%) completed the survey, and 89 were eligible to participate. Of those, 85 (96%) also completed round #2. Nine measures were chosen as 'top 5' in their respective category by over 50% of respondents (Table 1).

**Conclusion:** Our expert panel identified the most important transition measures as provider

communication, timing of the first adult clinic visit, patient self-efficacy and adherence to medical visits and treatment, trust and quality of life. Disease specific knowledge and medical outcomes including acute care utilization were not considered among the most important transition metrics. Using these results we can better develop and evaluate transition programs for young adults with SCD.

Table 1: Transition Quality Measures in the 'Top 5' of over 50% of Respondents and their Corresponding Feasibility Rating

	Measures in the 'top 5' of over 50% of respondents	Median Importance	Median Feasibility
Process Measures	Counseling about transition prior to transfer	9	9
	Written transfer summary being sent to adult provider	9	9
	Direct communication between pediatric and adult providers during transfer	9	8
	First visit to adult provider is within appropriate interval of leaving pediatric hematology	9	8
Patient Factors	Patient keeps clinic appointments	9	9
	Patient remains adherent to treatment and medications	9	7
	Patient has good self-efficacy (ability to manage their illness day to day)	8	6
Outcome Measures	Overall quality of life	9	7
	Patient trust in their adult provider	9	6

Poster # 577

## USE OF SIROLIMUS IN VASCULAR ANOMALIES DURING INFANCY

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**Background:** Sirolimus is an mTOR inhibitor that has found increased use in a variety of vascular malformations and tumors. The dosing is based on the serum level, with a usual starting dose of 0.8mg/m<sup>2</sup>/dose po twice daily and a goal of 10-15ng/ml. These settings were supported by a recent Phase II clinical trial on the use of sirolimus in complicated vascular anomalies. While dosing by body surface area (BSA) is preferable > 1 year of age, many of the affected patients are newborns (both term and premature gestations) or infants, and dosing of medication by weight or an adjustment of BSA formula might be preferable.

**Objectives:** To analyze the dose-trough level relation in the recent infants treated with sirolimus at our center and to suggest a different infant/neonate dosing range.

**Design/Method:** Five infants were included in the study: 3 of them received sirolimus for Kaposiform Hemangioendothelioma (all full term, ages 5 - 7 months at initiation of treatment). The other 2 received Sirolimus for lymphatic malformations: a) 34wGA started at 2-months-old for large cystic hygroma and head lymphedema; b) 26wGA started at 2.5-months-old for diffuse lymphatic malformation and chylothorax.

**Results:** All of them initiated the medication po twice daily and the dose was adjusted depending on the trough level. We noted that when using the 0.8mg/m<sup>2</sup>/dose (or even in one case at 0.55mg/m<sup>2</sup>/dose) the trough levels for the patients < 6-months-old were in the "toxic range" (as high as 16-26ng/ml), causing increases in liver enzymes and triglycerides, as well as multiple adjustments of doses and multiple blood draws. None of the babies displayed any clinical evidence of toxicity related to these levels, and all chemical abnormalities resolved when the doses were reduced and the drug troughs declined. We suggest using as initial dose

0.4mg/m<sup>2</sup>/dose po BID for patients < 6-months-old and then titrating up/down as needed.

**Conclusion:** Using sirolimus on a larger number of patients with an age span ranging from a few days old to adult years mandates a closer look at the pharmacology of the medication and appropriate adjustment of the starting dose.

Poster # 578

## **ENHANCED ANGIOGENESIS AND HEME-OXYGENASE-1 ACTIVITY IS ASSOCIATED WITH MELANOMA GROWTH IN SICKLE CELL DISEASE MICE**

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**Background:** The effect of sickle cell disease (SCD) on tumor growth is unclear. Sickled red blood cells may form aggregates within the microvasculature of tumors and reduce blood flow leading to impairment of tumor growth. However, there is a paucity of clinical data related to tumor growth in the SCD population.

**Objectives:** To determine how SCD impacts tumor growth and the mechanism(s) involved.

**Design/Method/Results:** SCD (n=10) and wild type (WT) (n=10) mice (generated by bone marrow transplantation) were injected subcutaneously (over lateral chest wall) with B16 melanoma cells. From day 1 to 21, tumor growth rate was nearly identical between the 2 groups, however from day 22 to day 29, tumor growth was accelerated in SCD mice compared to WT mice (p=0.018 for growth curves). At sacrifice (day 29), the weight of the excised primary tumor was increased in SCD compared to WT mice (1772 ± 295.0 vs 901.9 ± 131.8 mg, p=0.02). Since heme-oxygenase-1 (HO-1) has been shown to promote tumor growth and to be elevated in SCD, HO-1 activity was measured. Increased HO-1 activity was found in the liver (7.91 ± 1.49 vs 5.64 ± 0.94 pmol/mg protein/hr; p=0.0003), plasma (2.61 ± 0.63 vs 1.93 ± 0.48 pmol/uL/hr, p=0.016) and tumors (1.48 ± 0.19 vs 1.32 ± 0.12 pmol/mg protein/hr, p=0.04) of SCD compared to WT mice. To determine if SCD was associated with enhanced angiogenesis, which may promote tumor growth, matrigel plugs were injected subcutaneously into SCD (n=3) and WT (n=3) mice. 10 days later, plugs were excised and immunostained with anti-CD31 for quantitation of blood vessels. Matrigel plugs harvested from SCD mice contained more blood vessels compared to WT mice (83.0 ± 7.1 vs 38.6 ± 3.4 vessels per mm<sup>2</sup>, p<0.02).

**Conclusion:** Growth of melanoma tumors is potentiated in a mouse model of SCD and this effect is associated with enhanced HO-1 activity and angiogenesis. Further studies are underway to determine the causal role of HO-1 in promoting tumor growth in SCD mice.

Poster # 579

## **Treatment Strategies for Airway Hemangiomas**

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**Background:** Hemangiomas of infancy (HOI) are unique benign pediatric tumors of endothelial cells, characterized by an initial phase of rapid proliferation, followed by slow involution and

complete regression in most children. Approximately 60 % of HOI occur in the head and neck region. Airway hemangiomas can cause significant morbidity and mortality during the phase of rapid growth. Early recognition and treatment of these lesions is therefore of paramount importance.

**Objectives:** Patients with airway HOI who were treated with propranolol were reviewed to assess the efficacy of this treatment modality.

**Design/Method:** Patients with suspected airway hemangiomas were admitted to the hospital and underwent ENT evaluation of the airway by direct laryngoscopy prior to MRI of head and neck to establish extent of the hemangiomas. Baseline studies included electrocardiogram, echocardiogram prior to initiation of propranolol at a dose of 1mg/kg/day. Blood pressures and blood sugars were monitored. Dose of propranolol was increased to 2mg/kg/d on day 2. Patients were discharged from the hospital when cleared by ENT for airway safety and followed closely in the outpatient setting by the comprehensive vascular anomalies team.

**Results:** Eight patients with airway hemangiomas were treated at our institution over a period of 5 years. Three patients were treated with a combination of surgery, steroids and propranolol. Two patients needed emergency tracheostomy procedures to maintain airway patency. Five patients were treated with propranolol alone, ranging from doses of 2-3 mg/kg/day divided twice or thrice a day. Early response to propranolol therapy was noted in these patients with improvement in respiratory symptoms such as stridor within a week of initiation of therapy; none of these patients needed tracheostomy procedures. The average duration of treatment with propranolol was 12 months. 4 patients remain on therapy at this time, continuing to show significant improvement in respiratory symptoms. All the patients tolerated propranolol therapy well, without any complications.

**Conclusion:** We present a case series of infants with obstructive airway HOI who have been successfully treated with propranolol. Propranolol is a safe alternative to traditional invasive modalities such as tracheostomy and avoids exposure to steroids in this vulnerable population.

Poster # 580

## INFLUENCE OF HYDROXYUREA ON PICA IN SICKLE CELL DISEASE

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**Background:** Children with sickle cell disease (SCD) are prone to developing pica.

**Objectives:** The goal of this study was to observe the prevalence of pica in children with SCD, correlation of pica to severity of disease, and effect of hydroxyurea and regular blood-transfusion therapy on pica.

**Design/Method:** A retrospective chart review of patients between 2-21 years enrolled in the sickle cell center was performed. Disease type was determined by hemoglobin electrophoresis. Patients were considered to have pica if they gave history of eating nonfood substances at  $\geq 1$  visits. Disease severity was classified based on number of hospital admissions for vaso-occlusive crisis, acute chest syndrome and history of blood transfusions. Comparisons were made between patients with and without pica with hemoglobin-SS/S $\beta^0$  thalassemia and hemoglobin-SC/S $\beta^+$  thalassemia. Influence of hydroxyurea and regular blood-transfusion therapy on resolution/improvement of pica in patients with hemoglobin-SS/S $\beta^0$  thalassemia was observed.

**Results:** 32/108(29.6%) patients with hemoglobin-SS/S $\beta^0$  thalassemia and 19/71(26.8%) with

hemoglobin-SC/S $\beta^+$ thalassemia had pica (p=0.7). 25 patients with mild disease and 26 with moderate-severe disease had pica (p=0.84). Of 12 patients who received regular blood-transfusion therapy, 1(8.3%) patient had pica and 11(91.7%) did not have pica (p<0.002). Of 31 patients with hemoglobin-SS/S $\beta^0$ thalassemia who received hydroxyurea therapy, 10(32.3%) had pica and 21(67.7%) did not have pica. Spontaneous resolution/improvement of pica was seen in 6/21 patients with hemoglobin-SS/S $\beta^0$ thalassemia who did not receive hydroxyurea or regular blood-transfusion therapy and 13/19 patients with hemoglobin-SC/S $\beta^+$ thalassemia (p=0.01). 6/10 patients with hemoglobin-SS/S $\beta^0$ thalassemia and pica receiving hydroxyurea had resolution/improvement of pica versus 4/19 not receiving hydroxyurea or regular blood-transfusion therapy (p=0.036). Mean increase in hemoglobin-F was 19.8% in patients with resolution/improvement of pica and 4.6% in patients without resolution/improvement (p=0.06) after excluding non-adherent patients.

**Conclusion:** Prevalence of pica is similar in hemoglobin-SS/S $\beta^0$ thalassemia and hemoglobin-SC/S $\beta^+$ thalassemia. Pica does not co-correlate with the severity of the disease. Prevalence of pica is lower in patients receiving regular blood-transfusion therapy. Spontaneous resolution/improvement of pica is seen more often in hemoglobin-SC/S $\beta^+$ thalassemia. Hydroxyurea may help in resolution/improvement of pica in hemoglobin-SS/S $\beta^0$ thalassemia patients and increase in Hemoglobin-F could be the potential underlying mechanism. Alleviating the clinical course of hemoglobin-SS/S $\beta^0$ thalassemia may help in resolution/improvement of pica.

Poster # 581

## ORAL RAPAMYCIN USE IN PTEN HAMARTOMA SYNDOMES AND COMPLEX VASCULAR ANAOMLIES

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**Background:** The phosphatidylinositol 3-kinase/ Mammalian target of rapamycin (PI3-K/mTOR) signaling pathway is involved in vascular growth and organization. Loss of function of Phosphatase and tensin homolog (PTEN) or other upstream inhibitors of PI3-K/mTOR signaling can result in PTEN Hamartoma tumor syndromes associated with vascular anomalies (VA). Sirolimus inhibits mTOR and may therefore be useful in the management of patients affected by these syndromes and other complex VA.

**Objectives/Methods:** We conducted a retrospective chart review of pediatric patients treated with sirolimus for VA at our institution and report indications for initiating therapy, dosages, response and side effects.

**Results:** Five patients were treated. All had failed surgical and/or medical therapies or were facing debilitating or life-threatening complications. Starting dose was 0.8 mg/m<sup>2</sup>/dose, twice daily. Dose was titrated to a target serum concentration of 10–15 ng/ml. 16 yo female with Cowden's syndrome and growing, painful AVM's over the lower extremities confining her to a wheelchair. Pain resolved and patient was able to ambulate freely over the initial 11 months; however symptoms recurred while on treatment. 13 yo male with Bannyan-Riley-Ruvalcaba syndrome and a large progressive painful AVM in the abdominal wall. Pain resolved and was well controlled even during further AVM progression. 8 yo female with CLOVES syndrome and massive lymphatic malformation involving right upper extremity and hemithorax, growing

despite multiple surgical resections and associated with frequent episodes of DIC and sepsis. No further DIC or sepsis episodes were observed. Regained significant function of the affected arm. Improved quality of life. No further surgical interventions needed. 4 yo male with a retroperitoneal Kaposiform Hemangioendothelioma (KHE) associated with pain and Kassabach-Merrit Phenomenon (KMP). Pain and KMP resolved. 4 week old female with a rapidly enlarging VA of the neck (KHE vs hemangioma) with tracheal compression on MRI was started on sirolimus and propranolol. Tumor decreased by 50% in 2 weeks. Sirolimus was discontinued after biopsy confirmation of infantile hemangioma. The therapy was well tolerated. Hyperlipidemia was noted in 3 of 5 pts.

**Conclusion:** mTOR inhibitors may be useful in complex VA in children. Because of the rarity of these conditions, multi-center studies are required to confirm efficacy and determine optimal dosing

Poster # 582

## **ALTERED VENTRICULAR GLOBAL LONGITUDINAL STRAIN IN CHILDREN WITH SICKLE CELL DISEASE**

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**Background:** Patients with sickle cell disease (SCD) have increased risk of cardiopulmonary disease with subsequent higher morbidity and premature mortality. Decreased myocardial deformability (smaller absolute global longitudinal strain), is an early sign of myocardial dysfunction and may result from elevated pulmonary pressure or diastolic dysfunction. Few studies have investigated right and left ventricular global longitudinal strain (RVGLS and LVGLS) among children with SCD; none has investigated the role of disease-modifying therapies (hydroxyurea, chronic transfusions) on strain.

**Objectives:** 1) compare RVGLS and LVGLS in pediatric SCD with published normal reference values, 2) investigate the association of disease-modifying therapies and global longitudinal strain, 3) investigate the relationship between hemoglobin, lactate dehydrogenase, tricuspid regurgitation velocity (TRV), and global longitudinal strain.

**Design/Method:** Prospective measurement of RVGLS and LVGLS by speckle-tracking echocardiography in children with Hb-SS and HbS $\beta^0$  thalassemia ages 5-19 performed with central reading by one single cardiologist. Biomarkers of hemolysis were obtained concurrently.

**Results:** RVGLS was significantly reduced in children with SCD, but LVGLS was not (Table). Among children with SCD, 88% and 37% had absolute RVGLS and LVGLS values below the mean for normal children, respectively. RVGLS significantly decreased with age. No association was observed between RVGLS, disease-modifying therapies, hemoglobin, lactate dehydrogenase, or TRV.

**Conclusion:** Right ventricular deformability is decreased in children with SCD, is independent from TRV elevation, and may represent an early marker of pathologic cardiac change. Although no difference was observed in patients receiving disease-modifying therapies, longitudinal data are needed to determine their role in preventing deterioration of RVGLS.

Table. RVLGS and LVLGS in SCD Compared with Published Normal Reference Values

Right Ventricular Global Longitudinal Strain (%)										
	HbSS and HbS $\beta^0$ thalassemia				Reference Groups				P-Value (T-Test)	Reference
Age Group	N	Mean	Std	95% CI	N	Mean	Std	95% CI		
All Ages ( $\leq 19$ years-old)	85	-25.6	3.6	-26.4 to -24.8	226	-29.0	--	-31.5 to -26.5	--	Levy PT et al, J Am Soc Echocardiogr. 2014 May;27(5):549-60
					26	-34.1	5.0	-36.1 to -32.1	<0.0001	Blanc J, et al, J Am Soc Echocardiogr. 2012 May;25(5):511-7
Left Ventricular Global Longitudinal Strain (%)										
	HbSS and HbS $\beta^0$ thalassemia				Reference Groups				P-Value (T-Test)	Reference
Age Group	N	Mean	Std	95% CI	N	Mean	Std	95% CI		
$\leq 9$ years-old	31	-22.7	1.8	-23.4 to -22.0	45	-21.9	2.1	-22.5 to -21.3	0.0798	Kaku K et al, J Am Soc Echocardiogr. 2014 Jan;27(1):55-64
10-19 years-old	63	-21.9	2.4	-22.5 to -21.3	31	-21.4	3.4	-22.6 to -20.2	0.47	
All Ages ( $\leq 19$ years-old)	94	-22.2	2.3	-22.6 to -21.7	76	-21.7	--	--	--	

Poster # 583

## PHLEBOTOMY TO TREAT IRON OVERLOAD IN CHILDHOOD CANCER SURVIVORS

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**Background:** Iron overload is increasingly being recognized as a complication in survivors of pediatric malignancies. Intensive treatment protocols, hematopoietic stem cell transplant, and aggressive salvage therapies for relapse have contributed to the increasing use of transfusion support in pediatric oncology. Phlebotomy has long been used as a means of iron reduction in patients with iron overload. Phlebotomy is an inexpensive and accessible treatment compared to chelating agents. Although anecdotally used in pediatric cancer survivors, to our knowledge, it has not been reported for treatment of iron overload in the pediatric cancer survivor population. **Objectives:** To describe the experience of a tertiary care pediatric hospital using phlebotomy to treat iron overload in childhood cancer survivors.

**Design/Method:** A retrospective chart review of pediatric long-term cancer survivors from January 2008-December 2013 identified all children with a diagnosis of iron overload and who received phlebotomy. Ferritin values at the start and end of phlebotomy, total number of phlebotomies, adverse events during phlebotomy and reason for discontinuing phlebotomy were all recorded.

**Results:** A total of 4 patients with severe iron overload treated with phlebotomy were identified with a serum ferritin greater than 1000 ug/L (range 1146 to 1405 ug/L) prior to starting phlebotomy therapy. Patients received an average of 7.7 (range 4-10) phlebotomies at monthly intervals. The median ferritin at the last phlebotomy was 657 ug/L (range 451-1295 ug/L). Half the patients achieved the target ferritin of < 500 ug/L within 1 year of starting phlebotomy. No patient experienced any adverse effects during any phlebotomy.

**Conclusion:** Phlebotomy for the treatment of iron overload in this small group of childhood cancer survivors was well tolerated. However, after an average of 7 monthly phlebotomies, the median ferritin was still well-above normal range in all subjects. The utility of ferritin as a

screening tool is limited when underlying inflammatory states coexist and the greater availability of MRI Ferriscan may mitigate this concern. More research is needed to assess the effectiveness on other organs and burden on patients and families compared to the benefits of such therapy, as well as optimal screening methods for iron overload.

Poster # 584

## CONTRAST ENHANCED ULTRASOUND IMAGING TO DETECT ABNORMALITIES OF MICROVASCULAR BLOOD FLOW IN MURINE MODELS OF SICKLE CELL DISEASE

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**Background:** Microvascular dysregulation, abnormal rheology, and vaso-occlusion contribute to pathophysiology in sickle cell disease (SCD). Contrast-enhanced ultrasound (CEU) is a non-invasive perfusion imaging technique that has recently been applied to study SCD.

**Objectives:** In two separate genetically-modified murine models of SCD, skeletal muscle CEU was used to test whether there are differences in microvascular perfusion based on the known phenotypic differences in these models.

**Design/Method:** We studied: (1) NY1DD transgenic mice (n=18) (mild SCD without hemolytic anemia) and their wild-type controls (n=10); and (2) Townes mice (SS, n=6) (more severe SCD with hemolytic anemia) and their controls (AA, n=7). Quantitative CEU of the proximal hindlimb skeletal muscle was performed under resting normoxic conditions. Time-intensity data were analyzed to measure microvascular blood volume (MBV), microvascular blood transit rate ( $\beta$ ), and microvascular blood flow (MBF). Erythrocyte deformability was measured by elongation at various rotational shears.

**Results:** There was a non-significant trend for lower skeletal muscle MBF in NY1DD mice vs. controls ( $0.53 \pm 0.42$  vs.  $0.69 \pm 0.35$  ml/min/g;  $p=0.32$ ). This mild flow reduction was primarily due to a decreased  $\beta$  ( $0.27 \pm 0.13$  vs.  $0.38 \pm 0.15$  s<sup>-1</sup>,  $p=0.047$ ), whereas MBV was similar between both groups. In contrast, MBF in Townes SS mice at rest was higher than in AA controls ( $0.60 \pm 0.17$  vs.  $0.39 \pm 0.18$  ml/min/g,  $p=0.05$ ). This mild increase in MBF in SS mice was due to a greater  $\beta$  ( $0.36 \pm 0.08$  vs.  $0.16 \pm 0.05$  s<sup>-1</sup>,  $p=0.003$ ), yet there was also a trend toward lower MBV ( $0.03 \pm 0.01$  vs.  $0.05 \pm 0.02$  ml/g,  $p=0.09$ ). RBC deformability was decreased to a similar extent for all sickle cell groups compared to their respective controls, whereas only Townes mice had evidence for hemolytic anemia.

**Conclusion:** CEU revealed differences in the pattern of abnormal skeletal muscle perfusion in two separate murine models of SCD that reflect underlying differences in microvascular dysfunction. In the absence of hemolytic anemia, reduced erythrocyte deformability results in slowed blood microvascular transit rate. With more severe SCD with hemolytic anemia, there is a compensatory increase in muscle perfusion, yet MBV is reduced which may reflect either vaso-occlusion or a functional reduction in the number of microvascular units which has previously been associated with reduced nitric oxide bioavailability.

Poster # 585

## OVARIAN TISSUE CRYOPRESERVATION IS AN OPTION FOR FEMALE OF ALL AGES AT RISK OF INFERTILITY FROM GONADOTOXIC THERAPIES



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**Background:** There are now many conditions such as cancer and hematologic disorders, for which gonadotoxic therapy is used. The American Society for Reproductive Medicine (ASRM) and the American Society for Clinical Oncology (ASCO) recommend all patients be counseled about potential risk for infertility and fertility preservation options before starting any potentially gonadotoxic therapy.

**Objectives:** The objective is to present the experience of a single pediatric institution, Cincinnati Children's Hospital Medical Center, on a single fertility preservation technique: ovarian tissue cryopreservation (OTC).

**Design/Method:** An Institutional Review Board approved retrospective chart review was performed of all OTC procedures conducted at CCHMC between November 2010 and October 2014.

**Results:** Twenty-one female patients underwent OTC. Age range was 3 to 27 years (Mean = 12.5 years). Thirteen (62%) patients were pre-menarchal. There were 7 adult patients; one was married, and three were in relationships, one of which had a child. Of the patients who underwent OTC, there were 5 (24%) patients with solid tumors, 4 (19%) with neurologic tumors and 12 (57%) with hematologic disorders (such as Sickle Cell anemia, beta-thalassemia, etc.) requiring bone marrow transplant (BMT). All the patients were characterized as high risk for premature ovarian insufficiency (POI) from their planned therapy. All the patients diagnosed with a malignant tumor required immediate chemotherapy therefore OTC was their only option. The OTC procedure did not delay the treatment for any of the patients. The same surgical approach was utilized for all OTC: unilateral oophorectomy by laparoscopy. Laterality of the ovary removed for OTC was chosen by the gynecologist intra-operatively. Right oophorectomy was performed in 13 (62%) patients and left oophorectomy in 8 (38%) patients. Only one patient underwent more than one fertility preservation therapy: OTC and contralateral ovarian transposition. No complications were encountered from any of the procedures. One adverse event was encountered: thermal injury to fallopian tube.

**Conclusion:** The risk of infertility, regardless of severity, should be discussed with all patients/families before treatment/chemotherapy is started. Fertility preservation options, including OTC, should also be discussed and offered. We found that OTC, an experimental therapy, offers a viable fertility preservation option for both pre/postpubertal patients.

Poster # 586

## **PREVALANCE OF ELEVATED TRICUSPID REGURGITATION JET VELOCITY IN PATIENTS WITH SICKLING AND NON-SICKLING HEMOLYTIC ANEMIAS**

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**Background:** Adults with sickle cell disease (SCD) have an increased risk of developing tricuspid regurgitation jet velocity (TRV) elevation  $\geq 2.5$  m/s, which is associated with increased morbidity and mortality and markers of hemolysis. However, clinical consequences are unclear

in children and limited data are available in non-sickling hemolytic anemias (NOSHAs).

**Objectives:** Our goals were to validate the association between TRV and lactate dehydrogenase (LDH), to investigate the effect of treatment on TRV in SCD, and to explore the association of other biomarkers and TRV.

**Design/Method:** We enrolled children (<18 years) with SCD and children and adults with NOSHA. Laboratory data concurrent with echocardiogram were obtained.

**Results:** Children with severe SCD receiving chronic transfusions (CTXFN) had significantly higher TRV values than all other cohorts (Table). In children with severe untreated SCD, TRV was significantly associated with LDH, and hemoglobin, but not with arginine/ornithine ratio, total bilirubin, AST, or NTproBNP. In multi-variable analysis, only lower hemoglobin was associated with elevated TRV in children with severe untreated SCD.

**Conclusion:** Prevalence of elevated TRV in children with untreated severe SCD is similar to previous literature (30%). Children with severe SCD on CTXFN had the highest prevalence of TRV  $\geq 2.5$  m/s, possibly reflecting severity of disease. Our study validates the association between TRV and LDH only in severe untreated SCD. The lack of association between TRV and NTproBNP suggests this is not a useful marker to predict elevated TRV in these patients.

Table: TRV and biomarker results by cohort.

Cohort	N	Age (years)	TRV $\geq 2.5$ m/s (%)	LDH (U/L)	Hemoglobin (g/dL)	NTproBNP (pg/mL) <sup>1</sup>
Severe SCD, untreated (HbSS/HbS $\beta^0$ -thal)	117	8.1 (5.0-18.5)	29.6	582 (214-1315)	8.4 (6.7-11.2)	86 (8-804)
Severe SCD, Chronic Transfusion	29	12.9 (5.7-18.3)	57.1*	491 (211-1150)	9.5 (7.3-12.5)	50 (6-260)
Severe SCD, Hydroxyurea	76	13.2 (5.1-18.0)	34.2	450 (214-1354)	9.3 (6.9-12.5)	65 (9-316)
Non-severe SCD (HbSC, HbS $\beta^0$ -thal)	72	10.4 (5.2-17.8)	21.7	304 (157-811)	11.3 (10.0-14.9)	36 (6-266)
Pediatric NOSHAs (hereditary spherocytosis, pyruvate kinase deficiency, unstable Hb variants)	40	10.5 (5.1-17.5)	18.0	259 (154-2622)	12.3 (5.5-15.4)	75 (6-293)
Adult NOSHAs	21	39 (27.9-59.8)	27.8	185 (136-263)	13.9 (11.1-18.4)	42 (8-183)
	355					

Note: Results presented as medians (ranges) unless otherwise noted; <sup>1</sup>-N terminal brain natriuretic peptide, <sup>2</sup>- arginine/ornithine ratio  
\* Significantly higher than reference group (severe SCD untreated)  
Normal LDH 165 – 310 U/L, Normal NTproBNP < 160 pg/mL

Poster # 587

## TOBACCO USE BEHAVIORS AMONG SIBLINGS OF CHILDHOOD CANCER SURVIVORS: A REPORT FROM THE CHILDHOOD CANCER SURVIVOR STUDY

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**Background:** Having a sibling with childhood cancer may influence psychological and behavioral outcomes in adulthood. Moreover, some siblings of childhood cancer survivors demonstrate post-traumatic stress symptoms and adverse health behaviors including heavy and risky alcohol use behaviors. Tobacco use is a preventable health risk behavior associated with adverse health outcomes; however, tobacco use among siblings of survivors has not been systematically evaluated.

**Objectives:** To compare tobacco use among siblings of childhood cancer survivors with population-based estimates of use and to identify modifying factors for tobacco use.

**Design/Method:** Cross-sectional, self-reported tobacco use from 1,974 adult siblings of 5+ year

cancer survivors was compared to participants (n=24,105) in the 2007 National Health Interview Survey (weighted to match CCSS by age, race/ethnicity, household income, and education). Sociodemographic and cancer-related risk factors for sibling tobacco use were explored using multivariate logistic regression.

**Results:** Among siblings, 15.8% were tobacco users currently compared with 19.2% of NHIS participants and 23.7% were former smokers compared with 19.8% of NHIS participants. Compared to the NHIS participants, overall, siblings were less likely to be current smokers (Odds Ratio [OR] 0.77, 95% Confidence Interval [CI] 0.68-0.88), but no statistically significant differences were noted for former smoker status (OR 1.08, 95% CI 0.97-1.21). However, subsets of siblings were more likely to be current smokers including siblings with low educational attainment (OR 1.67, 95% CI 1.60-1.75) or income (OR 1.08, 95% CI 1.05-1.11) when compared to population rates with low education or income; respectively. Risk factors for current tobacco use among siblings included: household income <\$20,000 (OR<sub>adj</sub> 1.66, 95% CI 1.09-2.54), less than a high school education (OR<sub>adj</sub> 6.68, 95% CI 4.07-10.97), psychological distress (OR<sub>adj</sub> 5.36, 95% CI 2.21-13.02), and alcohol use (OR<sub>adj</sub> 3.68, 95% CI 2.50-5.41). Survivor diagnosis, treatment intensity, adverse health, and chronic health conditions did not impact current rates of smoking among siblings.

**Conclusion:** Overall, siblings of childhood cancer survivors do not appear to smoke at higher rates when compared to the general population. Despite this, efforts are needed to promote smoking cessation among those that are current smokers with particular emphasis among lower education or income populations.

Poster # 588

## **BMI AND THE ASSOCIATION WITH VASO-OCCLUSIVE CRISES IN PEDIATRIC SICKLE CELL DISEASE**

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**Background:** Children with sickle cell disease (SCD) have historically been underweight and with poor overall growth<sup>1</sup>. However, recent studies have demonstrated a trend toward overweight and obesity in pediatric SCD populations<sup>2</sup>.

**Objectives:** We aimed to investigate the comorbid effects of extremes of BMI (BMI <10% and BMI >95%) on frequency of hospitalizations for vaso-occlusive crises (VOC) in an inner city population.

**Design/Method:** This is a retrospective chart review study of patients with sickle cell disease who were admitted to St Christopher's Hospital for Children (SCHC) for VOC during a 12-month period. Study variables included: BMI, genotype, baseline hemoglobin, Vitamin D levels and concurrent disease modifying therapies. Their association with frequency of hospitalizations and duration of hospital stay was examined. The control group included patients with SCD followed in the Marian Anderson comprehensive sickle cell center that were not admitted to the hospital for VOC in the same 12-month period.

**Results:** During the study period, 328 patients, aged 0-22 years were evaluated. 110 patients were hospitalized to SCHC for VOC at least once, with 229 total episodes. Overweight and obese children constituted 19 % of hospitalized and non-hospitalized patients. Overall, BMI status does not influence rate of hospitalization for VOC (P=0.899) or duration of admission for VOC (P=0.653). Genotype of disease did not affect hospitalization rates (P=0.31) and within a

specific genotype of disease, BMI did not affect admission ( $P=0.578$  for HbSS,  $P=0.314$  for HbSC). Obesity is more associated with HbSC than HbSS ( $P=0.025$ ).

**Conclusion:** Our study does not demonstrate a significant association between extremes of BMI of patients and VOC severe enough to warrant hospitalization. There is an increasing trend toward obesity among the sickle cell pediatric population, reflecting the overall trend in childhood obesity in the 21<sup>st</sup> century. Further prospective and interventional research is required to define the effects of extremes of BMI on the severity of clinical disease phenotypes.

1. Barden, *J Pediatr*, 2000
2. Chawla, *Pediatrics*, 2013

Poster # 589

## **FATIGUE IN CHILDHOOD CANCER SURVIVORS: A REPORT FROM PROJECT REACH**

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**Background:** Fatigue is a debilitating side-effect of cancer and active cancer treatment. A well-established late effect of adult cancer survivors, the data remain inconclusive in studies of childhood and adolescent cancer survivors, especially those currently in adolescence and young adulthood.

**Objective:** To identify the prevalence and correlates of fatigue in survivors of childhood and adolescent cancer ages 12 years and older.

**Design/Method:** Participants were 268 CCS (Mean age 23 yrs; Mean age at dx 8 yrs; 52% female), enrolled in Project REACH, a longitudinal research study. Fatigue was measured using the PedsQL Multidimensional Fatigue Scale (MFS). Prevalence of fatigue was determined by identifying those with MDF scores falling 1 standard deviation below age-based means. For further covariate analyses, fatigue cases were defined as the lowest quintile on the MDF subscale of the PedsQL. Fatigue cases were compared to non-fatigue cases across demographic, treatment, quality of life, current health, and mental health variables. Measures included the PedsQL, SF-12, BDI-Y, and BSI-18.

**Results:** Thirty-seven participants (14%) reported fatigue, which is not significantly different from population norms. Univariate analyses demonstrated significant relationships between fatigue and age at study participation, household income, survival time, depression, and having  $\geq 3$  chronic conditions. Multivariable logistic regression excluding depression only identified having  $\geq 3$  chronic conditions as a significant correlate of fatigue (OR 4.3, CI 1.5-11.9). Analysis showed no evident association between fatigue and gender, age or treatment modality. Fatigued patients were significantly more likely to be depressed compared to non-fatigued patients ( $p<0.001$ ) and scored significantly lower across all of the PedsQL subscales measuring quality of life ( $p<0.001$ ). Of the 54 participants classified as fatigued, only 11 were depressed.

**Conclusion:** The prevalence of fatigue was lower than expected in this survivor population. Fatigue was highly correlated to having  $\geq 3$  chronic conditions, with the prevalence of conditions increasing with age. These findings may reflect advancements in cancer care aimed at reducing late-effects or delayed onset of late-effects in younger survivors. Fatigue also highly correlated with psychosocial well-being, underscoring the importance of fatigue assessment to promote optimal adjustment and QoL. Ongoing cohort evaluation will help better elucidate the evolution of fatigue in childhood cancer survivors.

## **HYDROXYUREA FOR NON-TRANSFUSION-DEPENDENT $\beta$ -THALASSEMIA: A META-ANALYSIS**

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**Background:** Non-transfusion-dependent  $\beta$ -thalassemia (NTD $\beta$ T) syndromes consist of  $\beta$ -thalassemia intermedia and moderate Hemoglobin (Hb) E/  $\beta$  thalassemias. They are characterized by varying degrees of chronic anemia and a wide spectrum of complications due to ineffective erythropoiesis and iron overload from chronic transfusions. Hydroxyurea (HU) is anticipated to decrease disease severity by raising Hb levels and reducing transfusion requirements.

**Objectives:** To examine the clinical efficacy and safety of HU in NTD $\beta$ T.

**Design/Method:** We searched MEDLINE, EMBASE, Cochrane library, ongoing trials registers, and major preceding conferences for randomized controlled trials and observational studies (sample size  $\geq 5$ ) that assessed HU in patients with NTD $\beta$ T. NTD $\beta$ T was sub-classified into mild and severe,  $<$  or  $\geq 4$  transfusion/year, respectively. The effect size was estimated as a proportion (responders over treated sample size). For Mild-NTD $\beta$ T, response rate (RR) was defined as a  $\geq 10\text{g/dL}$  increase in Hb. In Severe-NTD $\beta$ T, complete response rate (CRR) or overall response rate (ORR) defined as 100% or  $\geq 50\%$  reduction in transfusion need, respectively. All data was analyzed using Stata, Version 13.0.

**Results:** For Mild-NTD $\beta$ T, a total of 14 studies involving 344 patients met inclusion criteria. HU therapy was effective in raising Hb by  $10\text{g/dL}$  from baseline in 56% (95% CI, 46-67%). In Severe-NTD $\beta$ T, 8 studies involving 305 patients were included. HU was associated with a statistically significant decrease in transfusion need with CRR of 53% (95% CI, 37-70%) and ORR of 79% (95% CI, 68-91%). All of the studies had several limitations, such as small sample size, lack of comparison group, under-reporting of data and methods, and the majority having been observational studies. Adverse events were transient and improved with temporary cessation of the drug and/or adjustment of the dose.

**Conclusion:** HU appears to be well tolerated and effective in the management of NTD $\beta$ T (both mild and severe forms) by raising the Hb by at least  $10\text{g/dL}$  and decreasing the need for chronic blood transfusions completely or partially, respectively, in the majority of patients. Patients with NTD $\beta$ T may benefit from a trial of HU, though large RCTs assessing efficacy should be done to confirm the findings of this meta-analysis.

## **ARTERIAL STIFFNESS IN CHILDHOOD CANCER SURVIVORS**

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**Background:** Cardiovascular late effects occur earlier in childhood cancer survivors (CCS) than in the general population. Although previous studies have identified a small number of risk factors, it remains difficult to predict when or in whom cardiac late effects will occur. Arterial stiffness, as measured by Pulse Wave Velocity (PWV), has been shown in adults to be predictive of cardiac events and cardiac mortality. Increased PWV has been seen in adults following chemotherapy, but has not yet been explored in CCS.

**Objectives:** To evaluate PWV in a cohort of CCS and healthy children and assess independent risk factors for elevated PWV.

**Design/Method:** Carotid-femoral PWV was measured using applanation tonometry in CCS and healthy controls  $\geq 6$  years old. CCS were  $>12$  months off of therapy and free of cardiac disease, diabetes, and renal dysfunction. Data on height, weight, blood pressure (BP), and medications were recorded on all participants. Original cancer diagnosis, age at diagnosis, time off therapy, chemotherapy and radiation exposures were collected for CCS. Comparisons were done using t-test, chi-square and multiple regression analyses.

**Results:** Sixty-eight CCS (mean age  $17.3 \pm 6$  years, 52.9% male), and 46 controls (mean age  $19.1 \pm 5.4$  years, 30.4% male) were evaluated. CCS diagnoses included 34% lymphoma, 44% leukemia, and 22% solid tumor with 49% exposed to radiation. CCS were a mean of  $7 \pm 4.2$  years off-therapy. The two groups were statistically similar in age, BMI and BP. PWV did not significantly differ between CCS and controls ( $5.7 \pm 1.1$  vs.  $5.6 \pm 0.9$  ms,  $p=0.5$ ). CCS exposed to radiation therapy had significantly greater PWV than those not exposed ( $6.0 \pm 1.2$  vs  $5.5 \pm 0.9$ ,  $p=0.039$ ); however, the association did not hold after adjusting for age, SBP, and GFR. In the CCS group, PWV was directly associated with age, BMI, SBP, DBP, age at diagnosis and years off therapy, while PWV was inversely associated with GFR. Anthracycline dose and chemotherapy exposures were not predictive of increased PWV in CCS.

**Conclusion:** Contrary to published adult data, we did not identify increased PWV in CCS. Further studies are needed to determine the predictive value of PWV in this population and its utility as a screening modality.

Poster # 592

## HYDROXYUREA FOR $\beta$ -THALASSEMIA MAJOR: A META-ANALYSIS

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**Background:**  $\beta$ -thalassemia major ( $\beta$ -TM) is one of the most common inherited diseases worldwide.  $\beta$ -TM patients require life-long blood transfusions, resulting in iron overload with multi-organ morbidity and mortality. Hydroxyurea (HU), an oral chemotherapeutic drug, is anticipated to decrease the need for transfusions, either completely or partially by raising hemoglobin levels and thus decreasing the short and long term complications of chronic transfusions.

**Objectives:** To evaluate the clinical efficacy and safety of HU in  $\beta$ -TM patients of any age.

**Design/Method:** We searched MEDLINE, EMBASE, Cochrane library, ongoing trials registers, and major preceding conferences for randomized controlled trials and observational studies (sample size  $\geq 5$ ) that assessed HU in patients with  $\beta$ -TM. Primary end points of the study were complete and overall ( $\geq 50\%$ ) reduction in transfusion need. The effect size was estimated as a proportion (responders over the treated sample size) and reported as complete response rate

(CRR) or overall response rate (ORR) if they achieved 100% or  $\geq 50\%$  reduction in transfusion need, respectively. All data was analyzed using Stata, Version 13.0.

**Results:** A total of 10 observational studies involving 620 patients were included. HU was associated with a statistically significant decrease in transfusion need with CRR of 36% (95% CI, 23-50%) and ORR of 66% (95% CI, 52-79%).

All of the studies had several limitations, such as small sample size, lack of comparison group, under-reporting of data and methods, and being observational studies.

Adverse events (AEs) were transient and improved with temporary cessation of the drug and/or adjustment of the dose. No long-term AEs, including cancer or end organ damage were reported.

**Conclusion:** Hydroxyurea appears to be effective in the management of  $\beta$ -TM by decreasing the need for chronic blood transfusions completely or partially in a significant number of patients. It appears to be well tolerated and associated with mild and transient AEs. Patients with  $\beta$ -TM may benefit from a trial of hydroxyurea, though large RCTs assessing efficacy should be done to confirm the findings of this meta-analysis.

Poster # 593

## LOW PREVALENCE OF IRON OVERLOAD IN SURVIVORS OF CHILDHOOD CANCER

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**Background:** Liver dysfunction, cardiac dysfunction, endocrinopathy, and other complications have been described in heavily transfused patients, such as with sickle cell anemia or thalassemia, due to the significant amounts of iron present in therapeutic packed red blood cell (PRBC) transfusions. Many survivors of childhood cancer were heavily transfused during therapy, and previous studies have reported from 14% to more than 50% of survivors with iron overload.

**Objectives:** The primary goals of our research were to describe the prevalence of iron overload in survivors of childhood cancer at a single institution, and determine relationships between iron overload and different parameters of PRBC transfusion.

**Design/Method:** Participants were approached in the Thriving After Cancer (TAC) clinic at Rady Children's Hospital San Diego between July 2013 and June 2014. Ferritin level was obtained with other routine laboratory tests and serum ferritin level  $>500$  ng/mL was used to define iron overload. If detected, this was confirmed by repeat measurement along with inflammatory markers. In addition to basic descriptive and comparative statistics, correlations between ferritin and parameters of PRBC transfusions were performed.

**Results:** A total of 116 participants were recruited: 46% female, median 13 years of age at time of diagnosis, and median survival time of 6 years. Three of the 116 participants (2.6%) were found to have elevated ferritin levels in this previously unscreened population. All three patients with iron overload were diagnosed with malignancy in the teenage years, had received  $>8000$  mL total of PRBCs, and  $>100$  mL/kg of PRBCs. In the overall population, total PRBC volume transfused was most closely correlated with elevated ferritin values ( $r=0.74$ ,  $p<0.0001$ ). PRBC volume  $>8000$  mL was highly associated with elevated ferritin ( $p<0.0001$ ) with a positive predictive value of 75% and a negative predictive value of 100%.

**Conclusion:** There was an overall low prevalence of iron overload as assessed by ferritin elevation in contrast to previous reports. Risk of iron overload in this population was best

determined by total PRBC volume transfused, with a threshold of 8000 mL being highly predictive.

Poster # 594

## DIAGNOSIS OF UNSTABLE ALPHA CHAIN HEMOGLOBINOPATHIES IN THALASSEMIA INTERMEDIA

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**Background:** Thalassemia intermedia with non-deletional alpha-globin mutations usually leads to more severe disease than with deletional mutations. Diagnosis of non-deletional variants is missed by first line gap-PCR (polymerase chain reaction) and therefore requires alpha-globin gene sequencing.

**Objectives:** Describe the diagnostic approach to two siblings initially suspected to have alpha thalassemia trait who were found to have a rare unstable hemoglobinopathy in combination with the rightward (III) single alpha-globin gene deletion.

**Design/Method:** Genomic DNA was extracted from peripheral blood leukocytes and multiplex gap-PCR tests performed. Primers were designed to detect a single  $\alpha$ -globin gene deletion of rightward ( $-a^{3.7}$ ) and leftward ( $-a^{4.2}$ ) types and deletion of both  $\alpha$ -globin genes in the cis of the ( $--^{SEA}$ ), ( $--^{FIL}$ ), and ( $--^{THAI}$ ) types. The  $\alpha 1$ - and  $\alpha 2$ -globin genes were then amplified separately by PCR, and nucleotide sequencing performed to detect non-deletional mutations.

**Results:** Patient 1: A Filipino girl was referred to our hematology clinic at age 3 years with a history of microcytic anemia since birth (hemoglobin range 8.5-10g/dL). Heinz body preparation with brilliant cresyl blue stain for hemoglobin H bodies was negative. PCR detected a single alpha-globin gene deletion (-3.7 Kb, "rightward"). Nucleotide sequencing of the alpha-globin gene detected a missense mutation in the  $\alpha 2$ -globin gene codon 59 GGC>GAC or Gly59Asp (Hb Adana mutation).

Patient 2: Patient 1's brother presented at age 4 with microcytic anemia since birth (hemoglobin range 8-11g/dL). Heinz body preparation with brilliant cresyl blue stain for hemoglobin H bodies was negative. PCR and alpha-globin gene sequencing also revealed a single alpha-globin gene deletion (-3.7 Kb, "rightward") as well as the Hb Adana mutation in the  $\alpha 2$ -globin gene.

**Conclusion:** Accurate diagnosis of non-deletional alpha-globin mutations is important to uncover severe unstable variants like Hb Adana. Other double heterozygous or homozygous Hb Adana cases have presented with more severe anemia including hydrops fetalis. This case shows that Hb Adana can also present as more conventional mild to moderate Hb H disease, yet in other cases can lead to greater severity when paired with a different alpha globin deletion, leading to the attendant issues in antenatal genetic counseling.

Poster # 595

## EARLY HIGH-ACUITY HEALTHCARE UTILIZATION AMONG CHILDHOOD CANCER SURVIVORS

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**Background:** Increased rates of hospitalization among long-term survivors of childhood cancers have been observed when compared to peers with no history of cancer (1, 2). While long-term healthcare utilization and morbidity following cancer treatment have been studied, little is known regarding the patterns of healthcare utilization in the immediate time surrounding completion of therapy. This information may help predict future patterns of healthcare utilization as well as identify areas for early intervention and cost savings.

**Objectives:** To determine the number of emergency department (ED) visits and hospitalizations in the two years following completion of therapy among pediatric patients with either leukemia (ALL and AML) or lymphoma (Hodgkin and non-Hodgkin).

**Design/Method:** Using the Truven Health MarketScan administrative database, we identified a nationwide cohort of privately insured pediatric patients (0-21 years-old) who completed therapy for leukemia or lymphoma from 2001 - 2010. Patients were identified using ICD-9 codes in conjunction with billing codes for chemotherapy. End of treatment was defined using the last date on which IV chemotherapy was administered. Billing data also were used to identify subsequent ED encounters or hospitalizations.

**Results:** We identified 1100 patients who met study criteria, 633 (57.5%) with leukemia and 467 (42.5%) with lymphoma. The average age at the time of therapy completion was 9.5 years (SD 5.6) for patients with leukemia and 15.8 years (SD 4.3) for patients with lymphoma. Thirty-one percent of patients had an ED visit and 34% were hospitalized in the first year following treatment. In the second year following completion of therapy, 23% of patients were seen in an ED and 13% were hospitalized.

**Conclusion:** In a national cohort of privately insured patients undergoing treatment for either leukemia or lymphoma, nearly one-third experienced an ED visit and one-third underwent hospitalization in the first year following completion of active therapy. Analysis is ongoing to determine the reasons for these early, post-therapy high acuity healthcare encounters.

1. Kirchhoff, *Cancer Epidemiol Biomarkers Prev*, 2014
2. Kurt, *Pediatr Blood Cancer*, 2012

Poster # 596

## **PREDICTORS OF LIVER DISEASE IN PATIENTS WITH HOMOZYGOUS THALASSEMIA MAJOR ON BLOOD TRANSFUSION THERAPY**

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**Background:** Iron overload is the main cause of morbidity, especially from liver failure in patients with beta thalassaemia major (TM). Furthermore, blood transfusions increase the risk of blood born viral transmission with antecedent liver involvement.

**Objectives:** The aim of this study was to examine and evaluate the risk of different causes of liver disease in patients with homozygous thalassemia major on blood transfusion therapy.

**Design/Method:** We enrolled 80 children with TM (mean age $\pm$ SD, 9.5 $\pm$ 4.5; range 1.5–18 years) attending the pediatric day care unit for regular transfusional support in this retrospective cross-sectional study. They all were receiving packed red cells every 3–4 weeks. These patients were

prospectively treated either with oral iron chelator, Deferiprone at 100 mg/kg/ day in three divided doses or Deferasirox at 30-40 mg/kg/ day in single dose or with a combination of both drugs. They were monitored with blood counts, liver and renal functions monthly, serum ferritin once in three months and multi-transfusion virological screen once in six months. Liver iron load was also assessed by Liver MRI R2 values using a standardized protocol to determine the liver iron as mg Fe/dry weight.

**Results:** The average mean serum ferritin ( $\pm$ SD) was 1,334 ( $\pm$ 450) ng/ml, 1,308 ( $\pm$ 618) ng/ml and 1,967 ( $\pm$ 623) ng/ml in patients receiving DFP[n=32], DFX[n=28] and Combined treatment [n=20] respectively. None of the patients in this study cohort were found to have sero-converted with HIV, HCV or HbSAg positivity, although 3.75% and 1.25% were positive for anticore HBB and anti-HCV antibodies respectively. Iron overload remained the primary cause of hepatotoxicity with elevated mean serum ferritins above 1000 in 78% of the patients and mean Alanine amino transferase and Aspartate aminotransferase were 45.4 and 35.7IU respectively. Serum ferritin levels strongly correlated with Liver iron concentration (LIC) with a Pearson's coefficient of 0.514( $p<0.05$ ).

**Conclusion:** Although routine blood transfusions every 3-4 weeks remain the cornerstone treatment of homozygous thalassaemia major, suboptimal iron chelation remains the primary predictor of chronic liver disease in this patient population.

Poster # 597

## **A RARE CASE OF IDIOPATHIC CD4 LYMPHOCYTOPENIA WITH SECONDARY ANTIPHOSPHOLIPID ANTIBODY SYNDROME IN A YOUNG ADULT WITH EXCELLENT RESPONSE TO IMMUNOSUPPRESSIVE THERAPY WITH MYCOPHENOLATE MOFETIL**

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**Background:** Idiopathic CD4+ T cell lymphocytopenia (ICL) is a rare syndrome characterized by persistent CD4+ T cell lymphopenia ( $<300$  cells/mm<sup>3</sup> or  $<20\%$  of total lymphocytes) in the absence of infection with HIV-1 or other cause of immunodeficiency. Typically these patients present in middle age with opportunistic infections (OI) or auto-immunity. Anti-phospholipid antibody syndrome (APS) was reported in 2/39 patients in a prospective study.

**Objectives:** To report a case of ICL and secondary APS in a young adult treated with mycophenolate mofetil (MMF).

**Design/Method:** Case report.

**Results:** A 19 year old male with past history of atopic dermatitis, presented with Evan's syndrome, diffuse lymphadenopathy and bilateral nodular pulmonary opacities on chest computed tomography. His CD4 T-cell count was 41 cells/mm<sup>3</sup>. Anti-cardiolipin antibody immunoglobulin G (ACA-IgG), Diluted Russell's viper venom time (DRVVT) ratio and Anti-nuclear antibodies (ANA) were positive, but Anti-double-stranded DNA (Anti-dsDNA) was negative. Lymphocyte function assays revealed defective responses to antigens and mitogens. Lymphoma, autoimmune lymphoproliferative syndrome and infectious etiologies were ruled out and the patient was started on high dose steroids with a prolonged taper which stabilized his cytopenias but not the CD4 count. DRVVT ratio and ACA-IgG titers dropped, but remained positive. Pentamidine and fluconazole were started for OI prophylaxis. Five months after stopping steroids, thrombocytopenia returned, DRVVT ratio and ACA-IgG titers increased.

ACA-IgM and Anti-dsDNA became positive. Magnetic resonance imaging (MRI) of the abdomen showed areas in the left kidney consistent with infarcts. He was diagnosed with APS and treated with dexamethasone (40 mg/day for 4 days), MMF 2000 mg/day and warfarin. His platelet count normalized in 4 months, ACA-IgM became negative and ACA-IgG titer and DRVVT ratio decreased while Anti-dsDNA remained elevated. Kidney MRI after 6 months showed complete resolution of infarcts. The CD4 T-cell count was 265 cells/mm<sup>3</sup>, the highest since diagnosis, after 8 months of being on MMF. The patient has not had any other thrombo-embolic complications or OI so far.

**Conclusion:** This is a unique case of ICL and APS given our patient's young age and remarkable response to MMF. Further studies are needed to better understand the pathophysiology and guide effective therapy.

Poster # 598

## **EFFECT OF COMBINATION OF IRON CHELATION THERAPY IN HOMOZYGOUS BETA THALASSEMIA CHILDREN WITH HEAVY IRON OVERLOAD**

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**Background:** Optimal Iron chelation is today, the cornerstone for the successful management of patients with beta thalassaemia major (TM).

**Objectives:** The aim of this study was to compare combination iron chelation therapy of deferiprone (DFP) with desferrioxamine (DFO) or deferasirox (DFX) in beta thalassemia major patients with iron overload.

**Design/Method:** From amongst a cohort of 80 patients with homozygous thalassaemia major attending the paediatric day care unit for regular transfusional support, we enrolled 20 patients with TM (mean age $\pm$ SD, 11.1 $\pm$ 3.9; range 4–17 years) in this retrospective cross-sectional study who were receiving combination iron chelation therapy of deferiprone (DFP) with desferrioxamine (DFO) or deferasirox (DFX). 7 patients were receiving the combination of DFP+DFO for a median of 24 months; whereas, 13 patients were receiving the combination of DFP+DFX for a median of 20 months. They all were receiving packed red cell units every 3–4 weeks to maintain the pre-transfusion hemoglobin concentration above 9 g/dl. DFP was given at the dose of 70 to 75 mg/kg/ day in three divided doses and DFX at the dose of 30 mg/kg/ day in single dose whereas DFO was given at 40-60mg/kg as 8-10 hr subcutaneous infusion with a pump five days in a week. They were monitored with complete blood counts, liver and renal function assessment, at each monthly visit and serum ferritin once in three months and multi-transfusion virological screen once in six months.

**Results:** The average mean serum ferritin ( $\pm$ SD) at the start of combination therapy was respectively 2,823 ( $\pm$ 783) ng/ml, and 2,670 ( $\pm$ 530) ng/ml in patients receiving DFP+ DFO, DFP+ DFX combinations. The mean serum ferritin dropped from 2,823 ( $\pm$ 783) to 1,980 ( $\pm$ 564) in the patients receiving DFP+ DFO [ $p<0.05$ , student's t-test], whereas it dropped from 2,670 ( $\pm$ 520) to 1,707 ( $\pm$ 487) in the patients receiving DFP+ DFX combination [ $p<0.001$ , student's t-test]. The drop in Serum ferritin levels were negatively correlated with duration of combination chelation therapy [Pearsons correlation coefficient  $r=-0.29$ ,  $p<0.05$ ]

**Conclusion:** Combination iron chelation therapy of deferiprone (DFP) with desferrioxamine (DFO) or deferasirox (DFX) in beta thalassemia major patients with iron overload gives excellent results and was well tolerated in this patient cohort.

Poster # 599

## **AN EXTREMELY PALE TODDLER: TRANSIENT ERYTHROBLASTOPENIA OF CHILDHOOD IN A PATIENT WITH CONCOMITANT SICKLE CELL TRAIT DISEASE**

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**Background:** Transient erythroblastopenia of childhood (TEC) is an uncommon self-limiting benign red cell aplasia characterized by marked reticulocytopenia and normocytic, normochromic anemia. Unfortunately, there is a trend towards under-diagnosis of this condition; furthermore, there is a paucity of literature reporting the presentation of TEC in children with congenital red blood cell disorders making this a diagnostic challenge for the pediatric hematologist.

**Objectives:** We report the case of a toddler with TEC and concomitant sickle cell trait presenting with an unusual severe aplastic microcytic anemia.

**Design/Method:** Case report and literature review

**Results:** A 20 month old ex-33 week premature African American female, with failure to thrive and a questionable history of sickle cell disease presented with one week history of lethargy and malaise associated with a viral prodromal syndrome, characterized by subjective fevers, ear tugging, non-productive cough, and coryza. Physical examination was remarkable for marked pallor and a moderate systolic cardiac murmur; no hepatosplenomegaly, lymphadenopathy or rash were noted. As part of the initial work up a CBC revealed hemoglobin 2.2g/dL, hematocrit of 7.1%, low mean corpuscular volume of 70.2 fL and reticulocytes of 0.4%; peripheral smear analysis reported hypochromia, microcytosis, and polychromasia. Initially, the patient was transfused a total of 25ml/kg of packed red blood cells divided into aliquots of 5ml/kg each. Hemoglobin electrophoresis sent upon her admission demonstrated HbA 61.1%, HbA<sub>2</sub> 3.5%, HbS 33.6%, compatible with sickle cell trait and presumed thalassemia trait. Iron studies were normal and parvovirus PCR and serologies were negative. Post- transfusion hemoglobin level improved to 6.9g/dL; over the next few days hemoglobin remained stable and an increase in the reticulocyte count was also noted. Based on the transient nature of the anemia, a diagnosis of TEC superimposed to patient's baseline hemoglobinopathy was made.

**Conclusion:** To the best of our knowledge, this is the first report of a case of TEC in the setting of concomitant sickle cell trait. The presentation illustrates the diagnostic challenge on a patient with two different combined red cell disorders; clinicians should remain aware that in these cases patients tend to present with atypical findings, in order to prevent unnecessary therapeutic interventions.

Poster # 600

## **SUCCESSFUL MANAGEMENT OF BLUE RUBBER BLEB NEVUS SYNDROME (BRBNS) WITH SIROLIMUS**

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**Background:** BRBNS is a rare disease with vascular malformations in several systems of the body, most commonly skin and gastrointestinal tract. Bleeding from the gastrointestinal (GI) tract is a major complication, which may lead to chronic iron-deficiency anemia and need for frequent blood transfusions due to on-going gastrointestinal blood loss.

**Objectives:** To report successful management of BRBNS using Sirolimus as pharmacological therapy.

**Design/Method:** Case Report.

**Results:** A Hispanic female initially presented at three years old with a history of oropharyngeal bleeding since birth and skin vascular malformation. Upper and lower GI endoscopies were performed and revealed multiple vascular anomalies throughout the gastrointestinal tract. She was subsequently diagnosed with BRBNS. Due to chronic iron-deficiency anemia, GI bleeding, and increased need for blood transfusions, she underwent surgical removal of multiple blebs from her stomach, small intestine and colon. In addition, she had her right colon removed and is followed by Pediatric GI for continue GI bleeding and requirement for gastrostomy tube feedings. The patient is followed by Otolaryngology due to tracheostomy, conductive hearing loss and cholesterol granuloma. She underwent multiple sclerosing therapy to multiple lesions, including pericervical lesions. An Amicar trial (10 days) was not successful. Despite all the above, she remained anemic and required frequent blood transfusions, as often as every 1-4 months prior to initiation of Sirolimus therapy. Additionally, she was having black, tarry stools with bright red blood, 3-4 times a week. Daily Sirolimus therapy was initiated at dose of 0.05 mg/kg/dose and levels followed with a target range of 5-10 ng/mL. Since beginning Sirolimus therapy, there have been no reports of hematochezia or melena, and hemoglobin has remained above 11 g/dL. She is over 19 months into therapy and has not required any additional blood transfusions. No adverse drug reactions have been reported.

**Conclusion:** Sirolimus may be used in management of patients with BRBNS. Our case report shows resolution of GI bleeding and obviation of the need for multiple blood transfusions following initiation of Sirolimus therapy. To the best of our knowledge, this is the second report related to the use of sirolimus in a patient with BRBNS.

Poster # 601

## **PAROXYSMAL NOCTURNAL HEMOGLOBINURIA: NOT JUST APLASTIC ANEMIA - CASE REPORT**

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**Background:** Fourteen year old African American male referred after a blood test by the primary physician showed leukopenia and thrombocytopenia. There was no history of fever, cough, bone pain, weight loss, night sweats, oral ulcers, tiredness or weakness. Past medical history was significant for transient elevation of liver enzymes six months prior to this visit and leukopenia on previous occasions. He was treated for nocturnal enuresis with Oxybutinin.

**Test Results:** Complete blood count showed white cell count 2000/mcL, hemoglobin 12.7gm/dL, platelets 36,000/mcL, reticulocyte count 1.9%, LDH 1600U/L and undetectable haptoglobin. EBV titres suggested past infection and tests for hepatitis were negative. Bone marrow aspiration and biopsy showed decreased cellularity with no abnormal cells. Cytogenetics

and flow cytometry were normal with a negative DEB clastogen assay for Fanconi. FLAER testing revealed loss of GPI-linked markers including CD14, CD16, CD24, CD55 and CD59. Type III clone (complete GPI deficiency) and Type II clone (Partial GPI deficiency) were present suggesting a diagnosis of Paroxysmal Nocturnal Hemoglobinuria (PNH).

**Treatment:** Although asymptomatic and not transfusion dependent, eculizumab was started in view of the high risk of thrombosis and the presence of hemolysis as evidenced by undetectable haptoglobin and high LDH. After a month of treatment haptoglobin and LDH levels normalized.

**Conclusion:** PNH is a rare acquired clonal disorder of hematopoietic stem cells, related to a somatic mutation in the PIG-A gene characterized by a triad of recurrent intravascular hemolysis, thromboembolic episodes and variable degrees of bone marrow failure. The overall incidence is approximately 1/million, affecting both sexes with about 10% of patients diagnosed under the age of 21 years. Despite best supportive care, the 5 year mortality is 35%. The only potentially curative therapy for PNH is allogeneic bone marrow transplantation which is associated with significant morbidity and mortality. Eculizumab is the only safe and effective medical treatment available as evident from three multinational studies. It is a humanized monoclonal antibody that blocks activation of terminal complement C5 components. Treatment with Eculizumab improves survival and the quality of life by reducing transfusion requirement, improving constitutional symptoms and decreasing the incidence of thrombosis.

Poster # 602

## **MULTIFOCAL EPITHELIOID HEMANGIOENDOTHELIOMA OF THE LIVER AND LUNGS: TREATMENT WITH SIROLIMUS**

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**Background:** Epithelioid hemangioendothelioma (EHE) is a rare vascular tumor of intermediate malignant potential. It arises from the vascular endothelium of soft-tissue, bone and viscera. The tumor occurs most commonly after the second decade of life, mean age of 40 years. It has a variable course, and is often associated with multi-systemic localizations. Due to the rare frequency, an accepted standard of care has not been established. Therapeutic approaches include: observation, sarcoma-like therapy, radiotherapy, immunomodulators, surgical resection and liver transplantation. While the anti-angiogenic effect of mTOR inhibitors are used in the treatment of vascular tumors its use in EHE has been reported only in adults.

**Objective:** To describe a pediatric case with multifocal EHE treated with Sirolimus.

**Methods:** Case Report

**Results:** A 12 year old female was found to have multiple liver lesions on ultrasound during evaluation of acute abdominal pain. CT of the abdomen confirmed multiple liver lesions and demonstrated several other lesions in both lung bases. Laboratory investigations including CBC, liver enzymes, uric acid and LDH were within normal limits. Further laboratory investigations to rule out infectious or rheumatologic etiology proved negative. An ultrasound-guided liver biopsy confirmed the diagnosis of EHE. Further staging work-up with PET- CT and MRI, showed no brain or bone involvement, but multifocal disease in the liver and lungs. After considering various treatment modalities, we offered a trial of Sirolimus 2 mg BID (target level of 10-15 ng/mL). After 6 weeks of therapy her images were encouraging for good response and therapy was continued. Re-staging images after 3 months of therapy with Sirolimus showed improvement with calcification of some lesions. Patient continues to tolerate therapy well

without side effects. Her abdominal pain has subsided.

**Conclusion:** Sirolimus could be a reasonable option in the treatment of EHE in children. We propose that a trial of mTOR inhibition be considered as first option for the treatment of EHE presenting in childhood, prior toxic chemotherapy and/or other aggressive interventions. To our knowledge this is the first reported cases of a child with multifocal EHE treated with Sirolimus. Larger studies are needed to evaluate its efficacy in pediatrics.

Poster # 603

### **A CASE OF IRON-REFRACTORY IRON DEFICIENCY ANEMIA: A NOVEL MUTATION C.1807G>C (EXON 15) OF TMPRSS6**

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**Background:** Iron deficiency is a frequent cause of anemia (IDA) in infancy, and can be associated with various neuro-cognitive impairment. Iron-Refractory-IDA (IRIDA) has recently been described as an inherited cause of IDA due to loss-of-function mutations in the *TMPRSS6* gene, and characterized by lack of response to iron-replacement.

**Objectives:** To report a new case of IRIDA with its functional consequences, including neuro-psychological testing.

**Design/Method:** Blood count and biological parameters, including blood hepcidin level, were measured using commercially available kits. Complete neuro-psychological testing included Wechsler intelligence test and subtests (WPPSI-IV), and Kiddie continuous performance test and subtests.

**Results:** We report a five year old French Canadian male who was incidentally diagnosed with a severe microcytic anemia at two years of age (Hb 52 g/L, MCV 50 fL). He lacked physical signs and symptoms of anemia. While he showed some response to iron therapy (initially oral, followed by four intravenous Venofer injections), his hemoglobin level never rose above 92 g/L with MCV up to 60 fL, and he had persistently low serum iron with normal ferritin level. Blood hepcidin level was higher than his parents and control (patient: 11.2, father: 9.06, mother: 4.07 nM). Thorough evaluation, including normal bone marrow biopsy, was done. A compound heterozygosity for a paternally inherited *TMPRSS6* c.1324G>A and a maternally inherited and previously unreported c.1807G>C mutations were eventually identified. He had a normal development and growth. Neuro-psychological evaluation revealed excellent performance, with high WPPSI-IV scores (i.e 82th percentile for global intelligence, 97th percentile for memory scale).

**Conclusion:** *TMPRSS6* c.1807>C in conjunction with c.1324G>A results in IRIDA. In contrast to usual form of IDA, IRIDA may not be associated with neuropsychological deficits.

Poster # 604

### **A CASE REPORT ON THE USE OF PROPRANOLOL AS A SUCCESSFUL TREATMENT FOR PEDIATRIC VENOLYMPHATIC MALFORMATIONS (VLMS)**

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**Background:** VLMs are rare congenital malformations composed of a mix of low-flow vascular and lymphatic vessels. Treatments include surgical excision, sclerotherapy, and embolization although there is a high risk of local recurrence and complications, particularly cosmetic defects. For infantile hemangiomas (IH), propranolol is now adopted as first-line therapy. Propranolol is thought to cause down-regulation of the Raf mitogen-activated protein kinase signaling pathway, which reduces the expression of vascular endothelial growth factor (VEGF). Reducing VEGF inhibits angiogenesis, particularly in IH in the proliferative phase. There is some evidence of increased plasma [VEGF] in VLMs, with properties similar to IH in the proliferative phase. Propranolol causes marked reduction of plasma [VEGF]. Therefore, by reducing [VEGF], venolymphatic angiogenesis should be inhibited, presumably preventing VLMs from growing in the same manner that propranolol works on IH.

**Objectives:** We present a case of a 3-year-old male presented for admission with acute non-tender right facial swelling, with radiographic evidence of a 4.3 x 1 x 3cm, well-circumscribed, predominantly cystic and septated mass thought to represent a slow-flow VLM. Initial treatment options included surgical resection or sclerotherapy. Based on size, apparent rapid growth, and proximity to the right eye, a decision was made to find an alternative pharmacotherapeutic treatment. Oral propranolol was started at 1mg/kg/day for tolerance with a goal dosing of 2mg/kg/day. Serial physical exams showed a gradual decrease in size of the lesion, with no further signs by 3 months after starting treatment. A follow-up ultrasound verified complete resolution. Oral propranolol was discontinued, and the patient has remained well without signs of recurrence.

**Design/Method:** Case report.

**Results:** Literature review shows a paucity of publications on the use of propranolol in VLMs. Cases resulted in mixed results with either minimal or no response. Our patient was titrated up to the 2mg/kg/day standard dose used for IH and he showed clinical improvement with complete resolution by 3 months after starting therapy.

**Conclusion:** Considering the current safe use and efficacy of propranolol on IH, and its successful implementation in the treatment of our patient, propranolol should be considered as a treatment option for VLMs.

Poster # 605

## **PORTOMESENTERIC VENOUS THROMBOSIS AS FIRST PRESENTATION OF THROMBOPHILIC DISORDERS IN ADOLESCENTS**

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**Background:** Portomesenteric venous thrombosis (PMVT) is rare in adolescents and is usually associated with central venous lines or underlying inflammatory bowel disease, malignancy, or autoimmunity. Rapid recognition is essential but challenging in patients with no apparent predispositions.

**Objectives:** To evaluate the demographic, symptomatic, diagnostic, and therapeutic factors associated with adolescent PMVT.

**Design/Method:** We conducted a retrospective single-center chart review of adolescents diagnosed with PMVT between 2012 and 2014.

**Results:** Four patients (median age: 15 years) presented to the pediatric inpatient service with



four days to five weeks of abdominal pain with diarrhea and/or constipation, anorexia, and nausea. No patients had thrombosis histories, known thrombophilias, or predisposing conditions. After extensive diagnostic investigations, all patients' thromboses were ultimately discovered via imaging. The primary sites were superior mesenteric and portal veins, with two patients' thromboses extending to regional vessels. Two patients' thromboses were completely occlusive. All patients underwent standard inpatient anticoagulation. One patient additionally underwent thrombectomy and direct tPA thrombolysis and continuous infusion due to thrombosis extension. All patients were discharged on therapeutic anticoagulation upon at least partial thrombosis resolution. Throughout the median 11 month followup, no patients developed subsequent thrombosis extension, new unprovoked thromboses, or bowel necrosis. Patients underwent variable thrombophilia laboratory panels during hospitalization and followup. One patient had a durable protein S deficiency. Another patient was heterozygous for the factor V Leiden mutation, had a lupus anticoagulant (LAC) through four months after hospitalization, and had an isolated anticardiolipin antibody at four months. A third patient who smoked and had recently initiated estrogen therapy had a LAC during early hospitalization. A fourth patient who smoked had an intermittently positive LAC through five months and developed anti- $\beta_2$ -glycoprotein I antibodies at four months. All workups remained negative for predisposing non-hematologic conditions. **Conclusion:** PMVT can be the first presentation of thrombophilic disorders in previously healthy adolescents and may suggest presence of multiple thrombophilic laboratory abnormalities. As it is a rare diagnosis with significant complication potential, future work should standardize diagnostic approaches to subacute abdominal pain in adolescents to enable rapid thrombosis recognition, anticoagulation, and thrombophilia identification.

Poster # 606

## **RESPONSE OF BLUE RUBBER BLEB NEVUS SYNDROME TO SIROLIMUS TREATMENT: A SINGLE INSTITUTION EXPERIENCE**

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**Background:** Blue Rubber Bleb Nevus Syndrome (BRBNS) is a rare multifocal venous malformation syndrome involving predominantly the skin and gastrointestinal (GI) tract. BRBNS commonly presents in early childhood with anemia from GI bleeding. Modalities of treatment include corticosteroids, interferon- $\alpha$ , sclerotherapy and aggressive surgical resection. Sirolimus has been used for its antiangiogenic properties in several single case reports.

**Objectives:** This study reviews a single institution experience using sirolimus for the treatment of BRBNS.

**Design/Method:** We performed a retrospective review of 4 children with BRBNS who received sirolimus as part of their treatment regimens. A diagnosis of BRBNS was based on clinical, radiologic and pathologic criteria.

**Results:** Median age was 6.5 years (2-16 years). Pathologic evaluation revealed venous lymphatic malformation confirmed with Prox-1 staining in all patients. Patients received oral sirolimus with target drug levels between 8-10ng/ml. Responses to treatment were defined as: stabilization/decrease in size of skin lesions; resolution of transfusion requirements; reduction in pain. Median time to response was 1.5 months (1-3 months). Median follow-up was 12 months

(3-12 months). Lesion size and characteristics improved in all patients (Table 1). Two patients became transfusion independent. All patients reported significant decrease in pain and improvement in quality of life. One patient had resolution of coagulopathy. Side effects of sirolimus consisted of grade 1 mouth sores.

**Conclusion:** Sirolimus is safe and efficacious for the treatment of BRBNS. Further prospective studies are needed to evaluate the long-term effectiveness of this drug. In addition, this is the first report that identifies a lymphatic component as part of BRBNS.

Table 1 Sirolimus effect on 4 patients with BRBNS

Patient	Age	Location	Prior Therapy	Response to Sirolimus	Toxicity
1	16	Neck, Arms Left foot GI tract	Sclerotherapy LMWH	Change in morphology of lesions Decreased pain Improved coagulopathy LMWH discontinued	Grade 1 mucositis
2	10	Neck, Chest Back, Hip, Lower extremities, GI tract	Surgical excision	Decreased size of lesions, Decreased pain, Normalized hemoglobin	Grade 1 mucositis
3	3	Left shoulder Paraspinal muscles, Right knee, GI tract	Sclerotherapy Chronic transfusions	Change in morphology of lesions, Decreased pain, Normalized hemoglobin	Grade 1 mucositis
4	2	Neck Right shoulder, arm and hand	Chronic transfusions	Decreased size of lesions, Decreased pain, Normalized hemoglobin	Grade 1 mucositis

Abbreviations: GI: gastrointestinal. LMWH: Low Molecular Weight Heparin

Poster # 607

## HEMATOLOGIC CONCERNS ASSOCIATED WITH GOAT'S MILK ANEMIA

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**Background:** Goat's milk anemia was first noted in 1906, and later characterized as macrocytic and hyperchromic in the 1920's. Currently in America there is an emerging subpopulation of parents who are promoting raw goat's milk as a nutritionally viable alternative to formula or breastfeeding. For the purposes of educating the public and treating affected children, it is important to identify any serious associated morbidity.

**Objectives:** We describe a case of goat's milk anemia, including the non-hematologic sequelae.

**Design/Method:** The patient's medical record was reviewed for clinical presentation, course, and laboratory data.

**Results:** This case is a 5 month old unvaccinated male who presented with pallor and increased work of breathing. Physical exam showed a lethargic, pale-appearing infant with tachycardia and a 2/6 systolic ejection murmur. Significant initial lab findings were as follows: hemoglobin 2.7g/dL (MCV 97.6), platelets 67/ $\mu$ L, Na 161mEq/L, Cl 130mEq/L, BUN 73mg/dL, LDH 1886U/L, Uric Acid 11.7mg/dL.[GA1] A comprehensive dietary history revealed he was breastfed for one month, and then transitioned to pure, raw goat's milk from the family goat farm.

Due to critical lab values he was transferred to the intensive care unit, slowly transfused to a goal

of hemoglobin >8g/dl, and transitioned to infant formula with folic acid supplementation. Folate level later resulted at <2ng/mL. Iron and ferritin levels were normal. A femoral line was placed to allow slow infusion of red blood cells and adjust electrolytes. The infant subsequently developed a right common femoral vein thrombosis at his central line site. He was treated with low molecular weight heparin for 3 months with resolution of the thrombus. Follow-up 3 months after discharge showed normalization of labs and physical exam.

**Conclusion:** We report a case of goat's milk anemia involving severe anemia, hypercoagulable state, and severe electrolyte abnormalities with metabolic acidosis. These manifestations can all be attributed to the exclusive consumption of goat's milk, which leads to folate deficiency and a high solute load on immature kidneys. Our case highlights the importance of parental education and the related issues that need to be identified in the treatment of similar patients.

Poster # 608

## **VISCERAL LEISHMANIASIS ASSOCIATED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS: CASE REPORT**

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**Background:** A 14-year old previously healthy boy presented with intermittent high-grade fever, pallor, anorexia and weight loss for six weeks duration. He had been in Sudan for three years prior to his presentation to us in the sixth week of his illness. During the first five weeks of his illness in Sudan he was investigated and empirically treated for malaria, typhoid and brucellosis. His significant investigations there included negative bone marrow aspirate (BMA) with no parasites and hepatosplenomegaly on abdominal ultrasound.

**Physical Examination:** Examination revealed a tired looking boy with pallor, oral thrush, and petechiae on trunk. Positive systemic findings included a grade II systolic murmur and hepatosplenomegaly. Rest of his examination was unremarkable with no lymphadenopathy, edema or jaundice.

**Treatment Course:** Initial labs showed pancytopenia with reticulocyte count of 0.36%, ESR 67 mm/hr, LDH 2860 U/L, ferritin 5770 microgm/dl. Serology was negative for hepatitis A, B and C viruses, Epstein-Barr virus, Cytomegalovirus, Herpes simplex virus and human Immunodeficiency virus. Antibodies against Leptospira, Dengue, Brucella microti, CMV were negative. Malarial parasite peripheral smear and PCR negative. Bone marrow aspiration and biopsy was normal with no parasites seen. CT abdomen showed marked splenomegaly. Molecular studies were negative for primary HLH. Ceftazidime was started for febrile neutropenia with resolution of fever in four days. Keeping in account the fever, pancytopenia, splenomegaly, elevated ferritin and CD 25 (sIL-2R level of 5317 U/mL) a provisional diagnosis of HLH was made. He was started on high dose dexamethasone and discharged home with outpatient follow-up. He was re-admitted after three weeks with fever, pancytopenia, splenomegaly and a ferritin of 79500 microgm/dL when a repeat BMA was performed.

**Discussion:** Repeat BMA showed Donovan bodies suggestive of visceral leishmaniasis. In our patient the diagnosis of visceral leishmaniasis associated hemophagocytic lymphohistiocytosis was established after the fourth BMA. BMA establishes the diagnosis in significant number of cases but is often negative at onset due to the pauci-microbial nature of the disease and patchy

involvement. Repeated BMA, liver biopsy, blood cultures and serology may be required to establish the diagnosis. He was started on liposomal amphotericin with excellent clinical and hematological recovery.

Poster # 609

### **AUTOIMMUNE THROMBOCYTOPENIA IN A PATIENT WITH HUNTER SYNDROME: SHOULD IDURONATE-2-SULFATASE REPLACEMENT THERAPY STILL BE CONSIDERED?**

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**Background:** Hematological complications of Hunter syndrome (MPSII) are typically mild thrombocytopenia and neutropenia. There have been two reports of possible autoimmune thrombocytopenia associated with enzyme replacement therapy for mucopolysaccharidoses (Arylsulfatase B for MPSVI and Iduronate-2-sulfatase (Idursulfase) for MPSII).

**Objectives:** We describe a patient with Hunter syndrome who developed severe autoimmune thrombocytopenia and anemia while awaiting commencement of enzyme replacement therapy.

**Case Report:** A 4 year old male with Hunter syndrome presented with fever, pallor and fatigue. Initial investigations showed leucopenia (4600/ml,) anemia (Hemoglobin=5.3 g/dl), platelets 7000/ml and ANC 1940/ml. Viral testing for parvovirus B19, varicella, EBV, and CMV was negative. Blood and bone marrow studies showed pancytopenia, dyserythropoiesis, diminished iron stores, no malignant cells and no hemophagocytosis. Direct Coombs and antiplatelet antibody testing were positive. Treatment with Intravenous Immunoglobulin (IVIG, 1gm/kg x 4 doses) did not have a beneficial effect so oral prednisone 2.5mg/kg/day was commenced. The patient then developed acute intracranial and pulmonary hemorrhage necessitating oscillatory ventilation so plasmapheresis was commenced (continued for 11 days) and steroid dose was increased to intravenous methylprednisone (MP) 12mg/kg/da. With intensive supportive care, plasmapheresis and steroid therapy his platelet count increased to 94000/ml by day 3 and 195000/ml by day 13. Methylprednisone was weaned and stopped by day 45. On stopping the methylprednisone his platelet count slowly dropped to 147000/ml. In view of his previous intracranial and pulmonary hemorrhage, to prevent the platelet count from decreasing further intravenous methylprednisone was restarted (30 mg Q8h) and IVIG 4 mg/kg/day was re-administered for 4 days. When platelet count stabilized at about 150,000/ml without steroid therapy, the planned weekly Idursulfase replacement therapy was commenced. Over the next 3 months his neurological status returned to baseline and his platelet count has since remained stable over 150,000/ml

**Conclusion:** Contrary to previous case reports, in our patient therapy with Idursulfase did not precipitate/worsen autoimmune anemia or thrombocytopenia. Our experience would suggest that enzyme replacement may be safe in patients with Hunter syndrome and previous history of primary immune cytopenias.

Poster # 610

### **IS THERE AN AGREEMENT FOR POST-TRANSPLANT HEMOPHAGOCYTIC LYMPHOCYTOSIS DIAGNOSIS?**

**Background:** Hemophagocytic-Lymphohistiocytosis (HLH) results from excessive immune reactivation leading to heterogeneous clinical disorders. Despite the established guidelines, the diagnostic criteria for post-transplant HLH are not fully characterized.

**Objectives:** We assessed the inter-class correlation for degree of concordance between five guidelines models.

**Design/Method:** Through a single case and extensive PubMed literature search, using “HLH”, “Bone Marrow Transplant” and “Hematopoietic Stem Cell transplant (HSCT)” as key words, and limited the search to English and human subjects. Identified publications were reviewed by the authors. We only included cases with post-HSCT HLH diagnosis. Inter-class correlation for degree of concordance between five guidelines models was assessed via Cohen's Kappa coefficient.

**Results:** We report a 13 year-old male with relapsed AML, in second remission who underwent Bu/Cy conditioning, received 6/6 matched cord-blood stem-cell-transplant.

Tacrolimus/Methotrexate used for GVHD prophylaxis. HLH was suspected and treated based on early onset of symptoms and signs of fever, headache, skin eruption, neck and hip bone pain with no engraftment; associated with elevated CRP, serum ferritin and fibrinogen (day+7). By day +12 mild transaminitis, followed by persistent elevation of soluble IL-2 receptor and IL-6 were detected. 60 recipients with 61 post-HSCT HLH case reports, including our case were recognized, and compared according to Imashukr, Tsuda, Henter and Takakagi criteria (Table1). Case characteristics were: age range from 6 months to 69 years; 30 out of 54 males; cancer was the primary diagnosis for 50/60 cases. 54/61 received allogeneic-HSCT.

**Conclusion:** Objective lack of agreement between diagnostic models poses challenge for definition of HLH in this subset of patients.

Table: Post-Transplant HLH Guidelines Criteria Results

Diagnostic Criteria	Imashuku 1997	Tsuda 1997	Henter1991	Henter 2004	Takakagi 2009	Symptom met n/reported cases (%) [missed] <sup>a</sup>
Fever	**	**	**	**	Minor	56/61 (92%)
Cytopenia	**	**	**	**	Major	46/61 (75%)
Hyperferritinemia	**			**	Minor	46/60 (77%) [1]
BM involvement > 3%	**	**	(or other organs)	**	Major	49/60 (82%) [1]
Hepatosplenomegaly			**	**	Minor	18/57 (29.5%) [4]
Elevated TG/low fibrinogen			**	**		7/41 (17%) [20]
Low/Absent NK function				**		[61]
Elevated IL2 receptor				**		17/61 (28%)
Elevated LDH					Minor	20/30 (67%) [31]
Requirement	All four criteria	All three criteria	All five criteria	5/8 or molecular marker	2 major, or one major and 4 minor	Poor inter-class agreement of HLH diagnosis, with kappa of 0.068 (95% CI 0.01 – 0.12)
Guidelines Criteria Met <sup>b</sup> for HLH Diagnosis n/61 (%)	33/61 (54%)	40/61 (66%)	5/61 (8%)	13/61 (21%)	45/61 (74%)	

a) missed = not reported in the literature  
b) Criteria are not fulfilled if normal values, or not reported in the literature  
BM= bone marrow; IL=Inter Leukin

Poster # 611

## HERMANSKY PUDLAK SYNDROME: A CASE SERIES AND NOVEL MUTATION

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**Background:** Hermansky-Pudlak syndrome (HPS) is a rare autosomal recessive condition characterized by a bleeding diathesis and oculocutaneous albinism occurring in 1/500,000 to 1/million non-Puerto-Rican individuals. To date, there are eight identified genes associated with the disease that are involved with four protein complexes involving trafficking of lysosome related organelles. Patients also may experience neutropenia ranging from mild to severe, platelet function defects, pulmonary fibrosis and granulomatous colitis.

**Objectives:** In this case series, we aim to demonstrate a novel HPS mutation and describe the largest cohort of HPS patients at a single center.

**Design/Method:** We describe a series of five patients presenting with features consistent with HPS including demographic data, clinical features, family history, laboratory, and genetic testing by PCR amplification and next generation sequencing. In the patient with a suspected novel mutation, a classic family whole exome trio study was performed with extension to include her two healthy siblings to determine the causal HPS gene mutation.

**Results:** All five patients were of non-Puerto Rican Hispanic descent and presented with oculocutaneous albinism, neutropenia and easy bleeding. No other congenital anomalies were present. Four of the patients were siblings with AP3B1 (HPS2), c.2770\_delC mutations noted to

be homozygous in two children and heterozygous in the others. Genetic testing performed on the 5<sup>th</sup> patient revealed homozygous alterations in the HPS1, HPS4, and HPS5 genes, not previously described. Platelet dense granules were absent on electron microscopy. Family history revealed a male sibling with partial albinism and congenital neutropenia who died in infancy of severe pulmonary hypertension. Whole exome sequencing on the living affected proband, parents and siblings was performed to identify the causal pathogenic HPS mutation.

**Conclusion:** Although several new mutations have been implicated in HPS over the past several decades, patients with negative genetic testing and strong clinical suspicion suggest additional unknown pathogenic mutations remain. It is essential that new HPS cases are reported, particularly case series and suspected novel mutations, so that we may better understand and care for children with this rare and complex condition.

Poster # 612

## **ANAKINRA IN THE TREATMENT OF EXTRACUTANEOUS JUVENILE XANTHOGRANULOMA**

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**Background:** Juvenile Xanthogranuloma (JXG) is a non-Langerhans cell histiocytosis that usually presents with cutaneous accumulations of Birbeck granule-negative histiocytes. However, extracutaneous disease can occur and has a worse prognosis. Treatment includes vinca alkaloids and glucocorticoids. Anakinra, an interleukin-1 receptor antagonist, has been used to treat other histiocytic disorders by targeting the cytokine storm fundamental to these disorders' pathogenicity. The benefit of this therapy in JXG is unknown.

**Objectives:** We report two cases of extracutaneous JXG treated with anakinra.

**Design/Method:** Case series

**Results:** Patient 1 presented at 8 months old with respiratory distress and an infiltrate on chest x-ray. He worsened on broad-spectrum antibiotics. A chest CT revealed a large mediastinal mass with right pulmonary artery compression. After mass biopsy, the patient remained intubated and developed severe superior vena cava syndrome. Pathology results of the mass showed giant Touton-type giant cells that were CD15-, CD30-, and ALK- and CD68+, CD1a-, S100-, and Factor XIIIa+, consistent with JXG. He received high dose corticosteroids, vinblastine, and 10 days of anakinra with dramatic improvement. He is in remission after one year of treatment per Histiocyte Society LCH-III guidelines.

Patient 2 presented at 3 years old with fevers, arthralgia, and pancytopenia. He developed hepatosplenomegaly, pleural effusions, and anasarca. Bone marrow biopsy showed CD68+, CD1a-, and FXIIIa+ histiocytes. His symptoms worsened despite empiric treatment with corticosteroids, anti-thymocyte globulin, cyclosporine followed by prednisone, vinblastine, and etoposide. Re-evaluation in the setting of sepsis revealed lytic bone lesions that upon biopsy were CD68+ and Factor XIIIa+, consistent with JXG. Anakinra was initiated and continued for one year with near resolution of hepatosplenomegaly and pancytopenia. Six months after discontinuing anakinra, he relapsed and underwent elective umbilical cord hematopoietic stem cell transplant (HSCT). Two years later, he shows no evidence of recurrent JXG although has chronic complications from HSCT including gastrointestinal and skin graft-versus-host disease

and adenoviremia.

**Conclusion:** Extracutaneous JXG is a rare disorder with variable prognosis. These cases suggest that patients with severely symptomatic JXG may benefit from the addition of anakinra. Research is needed to explore the benefit of anakinra in the acute and maintenance phases of treatment for JXG and other histiocytic disorders.

Poster # 613

## **SUCCESSFUL TREATMENT OF CONGENITAL TTP WITH KOATE-DVI, AN INTERMEDIATE PURITY PLASMA-DERIVED FACTOR VIII CONCENTRATE**

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**Background:** Congenital Thrombotic Thrombocytopenic Purpura (CTTP) is characterized by the absence or dysfunction of the protease ADAMTS-13, leading to relapsing microangiopathic hemolytic anemia (MAHA). Typically CTTP is treated with fresh frozen plasma (FFP) or plasmapheresis. ADAMTS-13 is present as a contaminant in some purified Factor VIII products. We present the first case in the United States of a patient with CTTP treated with intermediate purity plasma-derived FVIII concentrate, and the first ever treated with Koate-DVI.

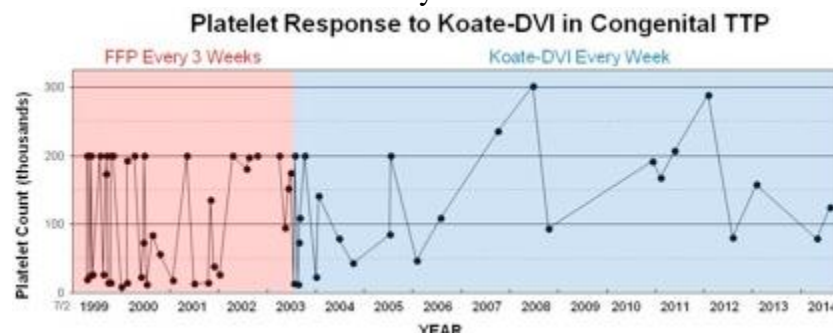
**Objectives:** We report a case of CTTP successfully treated with Koate-DVI over 11 years

**Design/Method:** Single case report

**Results:** A 6-year-old male was diagnosed with CTTP following 2 episodes of MAHA. ADAMTS-13 activity was 0% with no inhibitor present. For 4 years, the patient received regular infusions of FFP or solvent detergent pooled plasma (SDPP). Due to a shortage of SDPP in February 2003, the patient was treated acutely and then weekly with infusions of Koate-DVI at 30-40 units/kg/dose.

The patient experienced fewer hospitalizations (2) in the 50 months following induction of Koate-DVI therapy than in the 52 months preceding it (10). Platelet counts were significantly improved (**Figure 1**). Serum assays before and after treatment with Koate-DVI confirmed that ADAMTS13 activity normalized. The patient remains seronegative for Hepatitis B, C, and HIV, and has not developed an ADAMTS-13 inhibitor. Acute relapses have been successfully treated with Koate-DVI at 100 units/kg daily.

**Conclusion:** Prophylactic treatment with Koate-DVI may be an effective and safe therapy for CTTP and deserves further study.





## **HEPATOMEGALY WITH CHOLANGITIC ABSCESES WITH FAILURE TO THRIVE - A RARE CASE OF ISOLATED LIVER LCH AT PRESENTATION**

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**Background:** Langerhans cell histiocytosis (LCH) is a rare multisystem disorder, due to clonal proliferation of dendritic cells, affecting young children, with protean manifestations. Involvement may be single-system or multi-system. Liver involvement usually presents as biliary cirrhosis as part of multi-system disease.

**Objectives:** A case of isolated liver involvement without decompensation at presentation of LCH

**Design/Method:** Case-study

**Results:** A 13 months old female child without any prior co-morbidity presented with failure to gain weight since 4 months and frequent loose greasy stools since 2 months. She had no history of fever, respiratory complaints or abdominal distension. Examination revealed mild pallor, without jaundice or lymphadenopathy. Head-to-toe and skeletal examination were normal and she was at 50<sup>th</sup> percentile for weight. On systemic examination, liver was palpable 3 cm below right costal margin, with spleen tip just palpable. Remainder of the examination was normal. Labs revealed haemoglobin of 11.2 with total count of 23000 [55/45], normal peripheral smear with no abnormal cells; LDH was 900; total bilirubin of 1.6 mg/dL, direct fraction of 0.8 mg/dL, albumin of 3.5 g/dL and very high alkaline phosphatase (1680 IU/L); GGT was 851. Abdominal ultrasound revealed an enlarged liver (span 10 cm) with coarse echo-texture and multiple hypo-echoic lesions suggestive of cholangitic abscesses. Viral markers, Mantoux-test, HIV, Brucella-IgM were negative. Alpha-fetoprotein and immunoglobulin levels were normal. Liver biopsy showed enlarged biliary radicles with periportal inflammatory cell infiltrate suggestive of cholangitic abscesses, consistent with Caroli disease. Despite antibiotic treatment, there was no clinical improvement, counts remained high and lesions did not resolve. MRCP showed beading of biliary radicles, suggestive of sclerosing cholangitis. Intra-operative cholangiogram and open liver biopsy were consistent with Caroli's disease with cholangitic abscesses. However, immunohistochemical staining for S-100 in view of sclerosing cholangitis was positive. Skeletal survey, chest CT and bone marrow at diagnosis were negative for LCH. The patient was referred for chemotherapy and liver transplant.

**Conclusion:** Isolated, non-decompensated liver involvement by LCH is an extremely rare presentation and may mimic Caroli's disease/ syndrome with cholangitis. LCH should be considered in an infant with Sclerosing cholangitis. Such children with residual liver function may benefit from early liver transplantation.

## **METHOTREXATE PRECIPITATES VASO-OCCLUSIVE CRISES IN AN ADOLESCENT WITH SICKLE CELL DISEASE AND JUVENILE IDIOPATHIC ARTHRITIS**

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**Background:** Sickle cell disease (SCD) and Juvenile Idiopathic Arthritis (JIA) rarely co-exist. Due to similar symptomatology in each disease, the diagnosis of JIA is often delayed in patients with SCD. The treatment of JIA can be challenging in these patients.

**Objectives:** We report a rare case of a pediatric patient with SCD (1) who developed JIA as a teenager and (2) in whom methotrexate precipitated vasocclusive crisis (VOC).

**Design/Method:** Case report in a tertiary care pediatric hospital. Literature search performed on Pub Med using keywords "Sickle Cell Disease," "Juvenile Idiopathic Arthritis" and "Methotrexate."

**Results:** 15 year old girl with SCD developed multiple episodes of arthralgia and tenderness affecting her arms, elbows and ankles partially relieved by intravenous opiates and Ketorolac. After 7 months of repeated episodes, rheumatological evaluation resulted in diagnosis of rheumatoid factor positive polyarticular JIA (Cyclic citrullinated peptide antibody >250 units, Rheumatoid factor 39IU/L). Due to poor disease control with NSAIDs, she received treatment with prednisone and methotrexate. She developed arm pain typical of her VOC three days after her first methotrexate dose. One month later, she again presented with pain and noted that she had weekly episodes of VOC pain affecting her back and extremities with each dose of methotrexate.

**Conclusion:** JIA and SCD are rarely reported in association with each other. Literature search resulted in only 5 articles describing patients with hemoglobinopathies and rheumatologic disease. Each noted the difficulty in diagnosis of two diseases with similar symptomatology. In the presence of high disease activity and poor prognostic factors, methotrexate is often used as initial treatment for JIA. Our patient described VOC symptoms specifically noted after taking Methotrexate, which has not previously been reported in children. Increased awareness of the concomitant disease processes and the possibility of association between methotrexate and VOC will improve patient outcome and disease morbidity.

Poster # 616

## **GAMMA HEAVY CHAIN DISEASE IN ASSOCIATION WITH POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE: A CASE REPORT AND LITERATURE REVIEW**

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**Background:** Post-transplant lymphoproliferative disease (PTLD), often associated with Epstein-Barr virus (EBV) infection, occurs in about 6% cardiac transplant patients and is an important cause for morbidity and mortality. Gamma heavy chain disease ( $\gamma$ -HCD) is a rare disease of adulthood with approximately 130 cases reported in literature. Very little is known about this entity in children.

**Objectives:** To describe an unusual association of EBV-positive PTLD with  $\gamma$ -HCD.

**Design/Method:** Case report and review of literature

**Results:** An 11-year-old girl, who underwent an orthotopic cardiac allotransplantation at age 1.5 months, presented with fevers, weight loss, diarrhea, generalized lymphadenopathy and rising

EBV copy levels(223,000 copies/ml). One year prior, she was diagnosed with EBV-positive early PTLD of the soft palate, which responded to reduction of immunosuppression. Laboratory evaluation showed normocytic anemia with eosinophilia. Imaging studies revealed generalized lymphadenopathy and bilateral patchy pulmonary infiltrates. Upper and lower gastrointestinal(GI) and inguinal lymph node biopsies revealed PTLD with two components: an EBV-positive component with features resembling those seen in PTLD, early lesion, infectious mononucleosis-like, and an abnormal interfollicular plasmacytoid cell population that expressed IgG, lacked expression of light chains by in-situ hybridization and immunohistochemistry, and had a clonal immunoglobulin gene rearrangement, consistent with  $\gamma$ -HCD. Gastric biopsy demonstrated eosinophilic gastritis. Bone marrow aspiration and biopsy(BMaB) showed mild erythroid hypoplasia with increased eosinophils and minimal  $\gamma$ -HCD. Serum electrophoresis showed hypogammaglobulinemia. Urine protein electrophoresis revealed a small monoclonal kappa in the gamma fraction. She was treated with reduction of immunosuppression and 6 doses of rituximab (375mg/m<sup>2</sup>/week). Marked clinical improvement with resolution of the PTLD component on GI biopsies was noted. Repeat BMaB showed persistent  $\gamma$ -HCD component. She is currently six months post-rituximab therapy and doing well clinically.

**Conclusion:** There is no known reported association between EBV-positive PTLD and/or chronic immunosuppression and  $\gamma$ -HCD.  $\gamma$ -HCD is a lymphoplasmacytic neoplasm characterized by production of abnormally truncated IgG heavy chains without associated light chains. Often preceded by lymphoproliferative and autoimmune disorders, it is a heterogeneous disease with varied clinicopathologic presentations ranging from an asymptomatic transient process to a rapidly progressive and fatal form. Given its rarity, there are no treatment guidelines and therapy is aimed at the underlying lymphoproliferative process.

Poster # 617

## CELLCEPT TREATMENT IN RESISTANT ITP AND AUTOIMMUNE CYTOPENIA

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**Background:** Immune thrombocytopenic purpura (ITP) and Autoimmune Cytopenias, such as Evan's Syndrome, can be complicated by frequent exacerbations and remissions. The authors report four patients who proved to be refractory to frontline therapy, including steroids and intravenous immunoglobulin (IVIG), 6-12 months following diagnosis. All patients subsequently had successful stabilization of platelet counts with Mycophenolate Mofetil (CellCept). CellCept is a known immunosuppressant commonly used to prevent rejection in solid organ transplant patients.

**Objectives:** To describe the use of CellCept as a treatment option for pediatric ITP and Autoimmune Cytopenias refractory to frontline therapy.

**Design/Method:** The authors present four patients with refractory thrombocytopenia who responded to CellCept therapy (dose range 1000-1500mg/day). All patients demonstrated a response in platelet count within 1 month of treatment. The first patient, a 12-year-old male with Evan's syndrome who previously failed courses of steroids and IVIG, splenectomy, rituximab, mercaptopurine (6-MP) and vincristine, had platelet counts stabilize at normal levels following CellCept initiation. The second patient, a 14-year-old female with Autoimmune Cytopenia and CVID who previously failed steroids and IVIG, experienced normalization after initiating CellCept therapy. The third and fourth patients, a 19-year-old female and 15-year-old female

both with ITP refractory to steroids, IVIG, Rho (D) immunoglobulin, as well as Rituximab and 6-MP for the 19-year-old female, responded to CellCept with platelet counts returning to normal and maintaining stability.

**Results:** All four patients had normalization of platelet counts following CellCept. One of the patients with ITP has been weaned off of CellCept with continued normalization of platelets (18 months). The two patients with Autoimmune Cytopenias have not tolerated weaning CellCept, developing recurrent thrombocytopenia; however, both patients had platelet recovery once the dose was re-initiated. No significant toxicities have been experienced.

**Conclusion:** CellCept should be considered as a treatment alternative in patients who have refractory ITP and Autoimmune Cytopenia. This approach should also be considered in similar patients, responsive only to steroids, as a steroid-sparing agent.

Poster # 618

### **Near Fatal Presentation of Neuro-degenerative Langerhans Cell Histiocytosis**

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**Background:** Central Nervous system Langerhans Cell Histiocytosis (ND-LCH) is identified in two different forms. The well-known early presentation of infiltrative lesions involving usually the pituitary stalk presenting with diabetes insipides and the lesser known late presentation of Neurodegenerative LCH (ND-LCH) which is characterized by typical findings on imaging. ND-LCH usually has an insidious onset with cerebral and cerebellar degenerative signs such as neuro-cognitive decline, ataxia etc. and has a progressive course despite treatment.

**Objective:** We report a patient with ND-LCH who had a very rare fulminant presentation with brain edema and impending herniation resembling Hemophagocytic Lymphohistiocytosis of the CNS indicating an underlying basis of immune dysregulation and cytokine storm being a common feature to both diseases.

**Design/Method:** An 11 year old girl presented to the emergency with 3 week history of progressive headaches, diplopia and ataxia. She had a history of unifocal LCH of her left orbital region in 2008 and was treated with LCH3 protocol. Physical exam was remarkable for diplopia, papilledema and ataxia. Initial MRI brain showed diffuse pial enhancement of cerebellum and brainstem with focal areas of enhancement in the cerebellum with tonsillar herniation and obstruction to fourth ventricle. A comprehensive infective and autoimmune work up was negative. Cerebellar brain biopsy gave the diagnosis of ND-LCH with characteristic inflammatory T cell infiltrate and neuronal death. She was started on steroids, IVIG and cytarabine. She had a complicated course with brain herniation and had to undergo occipital decompression for stabilization. Subsequently she recovered slowly and at the time of discharge had minimal ataxia. She continues to get monthly IVIG and cytarabine.

**Results:** ND-LCH is a lesser known manifestation of LCH and characterized by progressive decline in function. Pathology involves inflammatory cells, particularly CD8+ T cells and very few or absent CD1a+ dendritic cells. These dendritic cells trigger an excessive inflammatory reaction with cytokines leading to a destructive process, the end result being neuronal death leading to a progressive unrelenting course of this disease.

**Conclusion:** This case presentation highlights a very acute and unique presentation of ND-LCH which can mimic HLH and can be fatal without appropriate management.

## ITP AS A RARE EXTRA-INTESTINAL MANIFESTION OF ULCERATIVE COLITIS

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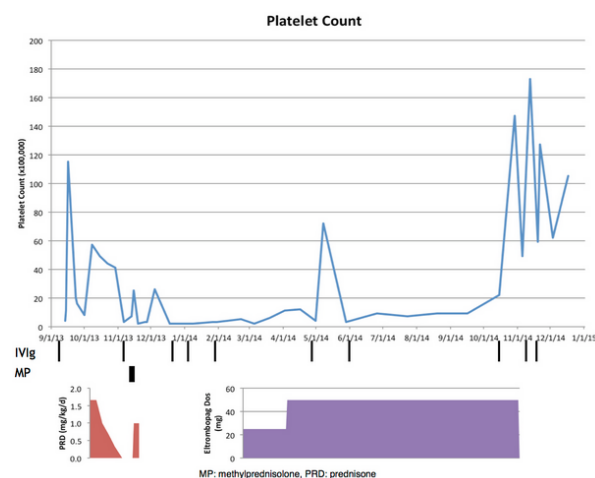
**Background:** Immune Thrombocytopenia (ITP) is a rare extraintestinal manifestation of ulcerative colitis (UC).

**Objectives:** Report a case of chronic ITP with subsequent diagnosis of UC

**Design/Method:** Case report

**Results:** A fourteen year old male diagnosed with acute ITP received IVIG (platelets 4000 $\mu$ L, WBC 4010/ $\mu$ L with a normal differential, and Hgb 11.5 g/dL). Three days after IVIG, platelets increased to 115,000/ $\mu$ L. However, three weeks later, he relapsed with bruising and thrombocytopenia. Over the following 16 months, despite treatment with prednisone, high dose methylprednisone, IVIG, and eltrombopag, clinically significant bruising and thrombocytopenia persisted, as summarized in the attached figure. Interestingly, he persistently had microcytosis without anemia. For this, he received ferrous sulfate. Over the next 16 months, he had two bone marrow evaluations which continued to support a diagnosis of ITP rather than other etiologies. While receiving eltrombopag, he demonstrated sustained clinical response despite poor hematologic response (platelets 4-22,000/ $\mu$ L). Eight months after starting eltrombopag, he developed abdominal pain with hematochezia. IVIG was administered and eltrombopag was held without improvement in stools. He was referred to gastroenterology and a colonoscopy demonstrated UC. Three weeks after initiating treatment with prednisone (1mg/kg) and mesalamine (2.4 grams BID), his platelet count improved to 105,000/ $\mu$ L. This response has now been sustained for 2 months.

**Conclusion:** Patients with ITP as an extraintestinal manifestation of UC respond poorly to traditional ITP treatments. The subsequent diagnosis and treatment of UC corrected his thrombocytopenia. Clinicians should consider inflammatory bowel disease in patients with poorly controlled ITP and microcytosis.



## LYMPHOMATOID GRANULOMATOSIS FOLLOWING PRIMARY EBV INFECTION IN A 14 YEAR OLD WITH TRISOMY 8 MOSAICISM

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**Background:** Lymphomatoid granulomatosis (LG) is a rare, EBV-driven, lymphoproliferative malignancy. It is most commonly diagnosed in immunocompromised patients, in the 5<sup>th</sup> to 6<sup>th</sup> decade of life, and has a male predominance. It usually presents with respiratory signs and symptoms, but can progress to multi-organ disease<sup>2-3</sup>. Clinical presentation is nonspecific; patients may have constitutional symptoms, cutaneous nodules, hepatosplenomegaly or meningeal signs. Lung imaging is also nonspecific, but cavitory lesions or parenchymal nodules can be seen. Given its rarity, there is often a delay in diagnosis. Furthermore, LG can mimic Wegener's granulomatosis histologically and radiographically<sup>2-3</sup>. Treatment is extrapolated from B-cell lymphoma protocols, such as R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone), and is based on histologic grading<sup>2-3</sup>. In lower grade LG, decreasing immunosuppression, when applicable, may be effective. Despite therapy, LG is often fatal with 5-year mortality of 63-90%<sup>2-3</sup>.

**Objectives:** We report a 14 year old female with Trisomy 8 mosaicism, repaired congenital heart disease and cardiomyopathy, diagnosed with LG after primary EBV infection.

**Design:** Case report

**Results:** She presented with infectious mononucleosis and developed progressive respiratory failure; multiple pulmonary nodules were noted on chest imaging. To our knowledge, this is the 50<sup>th</sup> child ever reported with this condition and the first with an underlying chromosomal abnormality<sup>2</sup>. Her lesions were Grade 3 histologically. She was treated with R-CVP (rituximab, cyclophosphamide, vincristine, prednisone), with two doses each of rituximab, vincristine, and a prolonged steroid course (anthracycline not used due to cardiomyopathy). She had progression of disease despite initial therapy. Directed radiation therapy to several pulmonary nodules and etoposide were added. Although her pulmonary lesions showed histologic evidence of involution with necrosis and her EBV viral load decreased, she developed widespread CNS lesions and died one month after diagnosis.

**Conclusion:** We postulate that her chromosomal condition predisposed her to malignancy. We also postulate that she may have had an undiagnosed immunodeficiency, causing her primary EBV infection to drive the development of LG, similar to patients with X-linked lymphoproliferative disorder<sup>1</sup>. Although rare, LG should be considered in the differential diagnosis of persistent pulmonary lesions of unclear etiology, especially in immunocompromised patients. Early biopsy may aid in diagnosis and enable prompt therapy.

Poster # 621

## LABORATORY DIAGNOSIS OF BERNARD-SOULIER SYNDROME: USEFULNESS OF PARAMETERS FROM THE SYSMEX-XE SERIES OF AUTOMATED CBC ANALYZERS

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**Background:** Bernard-Soulier Syndrome (BSS) is a rare (1:1000000) autosomal recessive inherited platelet disorder characterized by giant platelets, thrombocytopenia and mild to moderate bleeding diathesis. Due to its rare nature, the initial presentation of these patients may be to their primary care physician, and not to a hematologist who would be more familiar with BSS. As a result, initial investigations will likely consist of routine laboratory tests, including a complete blood count (CBC). The Sysmex XE-series CBC analyzers have the capability to quantify reticulated platelets on a routine CBC sample. The Immature Platelet Fraction (IPF) is identified by flow cytometry techniques and the use of a nucleic acid specific dye in the reticulocyte/optical platelet channel.

**Objectives:** To describe the CBC findings on the Sysmex XE-series CBC analyzers including the IPF, in a case of BSS.

**Design/Method:** All CBC data, including Sysmex analyzer information, was reviewed on a patient who was ultimately diagnosed with BSS.

**Results:** 20 CBC and peripheral blood smears were reviewed. Mean platelet count was  $54 \times 10^9/L$  (range 22 – 85). In 3/20, the analyzer was unable to report a platelet count, and a visual platelet estimate was reported. In all instances, the analyzers were unable to report a Mean Platelet Volume (MPV). IPF was available in 19/20, and was markedly elevated with a mean of 33% (range 24-45%). Review of the peripheral blood smears demonstrated an easily identifiable population of megathrombocytes, though this was not consistently reported by technical staff.

**Conclusion:** The IPF has been used to separate thrombocytopenic states into hypoplastic states (low IPF; e.g. chemotherapy, aplastic anaemia) vs. peripheral destruction (high IPF, e.g. ITP, TTP). In our case of BSS, the marked elevation in IPF correlates with the postulated mechanism of thrombocytopenia – that of decreased peripheral survival, necessitating compensatory bone marrow response. Familiarity with the CBC features of BSS (moderate thrombocytopenia, markedly elevated IPF, and inability to report a MPV) may help to separate out this disorder from other more common causes of thrombocytopenia presenting in childhood.

Poster # 622

## **CONCOMITANT PRESENTATION OF HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS AND POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE-LIKE LYMPHOMA IN A PRE-B-CELL ALL: AN UNUSUAL ASSOCIATION**

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**Background:** Hemophagocytic Lymphohistiocytosis (HLH) is a rare multisystem hyperinflammatory syndrome that is due to cytokine activation from overly stimulated lymphocytes and macrophages. Diagnosis is made based on specific criteria including clinical signs and laboratory abnormalities. However, many of these features are non-specific and the diagnosis of HLH can be often challenging, and prognosis is usually poor. EBV disease and its associated post-transplant lymphoproliferative disorder (PTLD) is more frequently seen when primary EBV infection occurs following bone marrow or solid organ transplant in children. Co-occurrence of both entities is rare.

**Objectives:** We report a case of HLH in a 4 year-old girl with pre-B-ALL, complicated by an aggressive unusual lymphoproliferative disorder.

**Design/Method:** Case report with review of medical literature.

**Results:** A 4-year-old Caucasian female with pre-B-ALL in remission, on maintenance therapy

presented to a tertiary care hospital with fever, pancytopenia, transaminitis and hepatosplenomegaly. CT of the abdomen revealed multiple new echogenicities in liver, spleen and both kidneys. Bone marrow aspirate examination showed no evidence of relapsing leukemia, with scattered histiocytes demonstrating features of active hemophagocytosis. Work-up revealed active EBV infection, hypofibrinogenemia, with elevated ferritin, triglycerides and IL-2 receptor. Patient was started on weekly dexamethasone and Rituximab for suspected EBV-associated HLH. Biopsy from the kidney lesions, however, showed the presence of clonally-restricted EBV associated 'PTLD-like' B-cell lymphoproliferative disorder, kappa-light chain restricted, that is associated with the presence of hemophagocytic syndrome observed in the bone marrow. Chemotherapy was started with cyclophosphamide, Rituximab and prednisone, however patient did not improve clinically and kidney lesions progressed. Third line therapy was attempted with Etoposide and Cyclosporin. Patient developed multi-organ failure and passed away. Autopsy revealed diffuse nodules involving the liver, spleen, kidneys and lungs with microscopic evidence of lymphoproliferative process.

**Conclusion:** EBV-associated PTLD is rare and fatal. Index of suspicion for PTLD was low, given that there was no history of bone marrow transplant or underlying immunodeficiency. The association of HLH with EBV-related PTLD-like lymphoproliferative disorder is rare. To the best of our knowledge, this is the first patient reported with this presentation. We present this case to highlight this unusual association so that this fatal disease is promptly addressed.

Poster # 623

## **MERCURY POISONING AS AN UNUSUAL CAUSE OF IMMUNE THROMBOCYTOPENIC PURPURA**

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**Background:** Without a high index of suspicion, acute mercury poisoning is often not included on the differential diagnosis for immune thrombocytopenic purpura (ITP).

**Objectives:** To describe the unique case of a patient who presented with ITP as the initial sign of mercury toxicity.

**Design/Method:** Case Report

**Results:** A previously healthy 7-year-old-male presented to the emergency department with increased bruising, wet purpura of the mouth and hematuria for two days. Initial labs revealed a normal WBC of  $10.7 \times 10^3/\mu\text{L}$  (61% neutrophils, 38% lymphocytes), hemoglobin of 11.1 g/dL and low platelets of  $7 \times 10^3/\mu\text{L}$ . The patient was subsequently diagnosed with ITP and treated with IVIG and prednisone with recovery of platelets to  $221 \times 10^3/\mu\text{L}$ . The patient was seen in hematology clinic for follow up three weeks later and found to have an elevated heart rate of 126 and blood pressure of 190/100. Physical examination revealed a restless appearing, diaphoretic male with mild swelling of his hands and feet, an intensely pruritic generalized pink rash and abdominal pain. Repeated testing on admission showed a normal platelet count and thyroid function tests. While pursuing work up for a possible catecholamine-secreting tumor, two other children from the same household presented with similar symptoms. Suspicious for environmental exposures, 24-hour urine collection for mercury was then obtained showing elevated levels in all three children. The source, on later investigation, was a mercury-containing sphygmomanometer that the patient found in the garage and broke in his carpeted bedroom. Markedly elevated levels of mercury vapor were found in the bedroom with lower levels



detected throughout other rooms of the house. The three children were started on succimer for chelation and placed on anti-hypertensive medications with slow improvement. The house was decontaminated and the family was allowed to move back into their home before any additional inhabitants were affected.

**Conclusion:** Although not well-described in adult or pediatric populations, mercury causes increased autoimmunity that can manifest as ITP. The pathophysiology is not completely understood, but a TH2 driven mechanism has been supported in mice models.<sup>1</sup> Timely recognition of this environmental risk factor during diagnosis of ITP is critical in providing correct treatment and preventing additional exposures.

<sup>1</sup>Vos, *Immunopharm. Immunot.* 2007

Poster # 624

## **ESTHESIONEUROBLASTOMA: A VERY RARE CAUSE OF A NASAL MASS IN AN 18-YEAR OLD FEMALE**

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**Background:** Esthesioneuroblastoma is an infrequent malignant tumor of the olfactory placode. Symptoms are related to the size and spread of the tumor. It commonly presents non-specifically with nasal obstruction, epistaxis and hyposmia. We present the case of an 18-year old female with esthesioneuroblastoma.

**Objectives:** Case describes the diagnosis and management of esthesioneuroblastoma.

**Design/Method:** Case report and literature review

**Results:** The patient is a previously healthy 18-year old Haitian-American female. Two months prior to presentation she developed gradual headaches, mild right eye proptosis, recurrent epistaxis and hyposmia. She had received right nasal cauterization to stop the epistaxis on one occasion. Neurological exam remarkable for anosmia but was otherwise normal. A MRI demonstrated a mass arising from the nasal septum and middle/superior turbinates resulting in complete obstruction of the right nasal passage. The lesion infiltrated the right medial rectus and extended superiorly along the anterior cranial fossa bilaterally. Her PET scan showed radioactive tracer collection bilaterally in the cervical lymph nodes. A biopsy was performed and the final pathology report showed tumor cells were positive for: synaptophysin, S100, CD66, T63 and NeuN1; negative for CD99, CD45, keratin, desmin and neuroblastoma antigen, indicative of olfactory neuroblastoma (esthesioneuroblastoma). Given the extent of disease and involvement in her cervical lymph nodes, the patient was classified with Kadish system Stage D tumor. She was treated with 3 cycles of neo-adjuvant chemotherapy with Cisplatin (80 mg/m<sup>2</sup> day 1) and Etoposide (100 mg/m<sup>2</sup> day 1-3). Interestingly, the repeat PET and MRI showed a near complete response of the tumor. She underwent unilateral neck dissection, bi-frontal craniotomy, and orbitotomy for tumor resection. Extensive tumor necrosis was noted at the time of surgery. She is currently receiving radiation therapy and weekly low-dose Cisplatin chemotherapy (30mg/m<sup>2</sup>) as an outpatient.

**Conclusion:** Esthesioneuroblastoma (ENB) is a rare, aggressive tumor with no established treatment in children and adolescents. This condition should form part of the differential diagnosis of a patient presenting with a nasal mass. Furthermore, this was a particularly

aggressive presentation of this tumor with a response to multi-agent chemotherapy that has been significant.

Poster # 625

## **FAMILIAL ERYTHROCYTOSIS AND HYPOXIA INDUCIBLE FACTOR 2-ALPHA**

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**Background:** Primary polycythemia is a rare condition to be diagnosed during childhood. Advances in molecular and genetic techniques have shed light on mutations causing disruption of the oxygen-sensing pathway that regulates the erythropoietin gene, raising awareness of these. Prompt identification and treatment are imperative to prevent symptoms with the goal of decreasing morbidity that may arise from the hyperviscosity.

**Objective:** Report of a case of a child presenting with erythrocytosis.

**Design/Method:** Case report

**Results:** A 4y/o Puerto Rican male was brought in for evaluation to the hematology clinic after an incidental finding of polycythemia on CBC. This was done after he presented with recurring headaches not associated with loss of consciousness, emesis, or any other neurologic findings. Past medical history was non-contributory. He was born full term and acquired development milestones appropriately. Family history was notable for paternal polycythemia treated with scheduled phlebotomy. Initial evaluation at the time showed hypertension for age, plethoric appearance, injected conjunctiva, and violaceous appearance of lips. The rest of his physical exam was found to be within normal limits. Initial CBC done in clinic showed polycythemia with hemoglobin level of 22.9g/dL and a hematocrit of 70.8%, rest of the cell lines were within normal parameters for age. Further workup to rule out cardiac, renal, as well as malignancy did not identify a cause for the polycythemia. Patient was recommended to have scheduled phlebotomy in order to alleviate symptoms, to which he was responsive. Phlebotomies improved his quality of life. Based on family history, his serum was sent to be tested for familial erythrocytosis mutation, and he was found to have a mutation on the hypoxia inducible factor 2-alpha (HIF-2 $\alpha$ ).

**Conclusion:** Primary polycythemia due to a mutation on HIF-2 $\alpha$  is a rare diagnosis in childhood. High index of suspicion and thorough family history will aide in prompt diagnosis and initiation of phlebotomy treatment to improve quality of life and possibly increase survival rate.

Poster # 626

## **SYNCHRONOUS TYPE I $\alpha$ AND TYPE II PLEUROPULMONARY BLASTOMA IN A CHILD WITHOUT DICER1 SYNDROME**

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**Background:** Pleuropulmonary blastoma (PPB) is rare, but the most common primary pediatric malignancy of the lung. It comprises 0.5% of all childhood malignancies and is associated with DICER1 mutation syndrome in 60% or more of cases. There are three morphologic stages which reflect the age at diagnosis and clinical outcome: Type I cystic, Type II mixed cystic and solid, and Type III solid. Types II and III have a poorer prognosis due to higher risk of relapse and metastases to the brain and bone. Types II and III are thought to evolve from Type I. Type Ir designates a cystic lesion with either regression or failure of tumor progression. There are limited examples of multiple types of PPB diagnosed synchronously in a child

**Objectives:** We report the synchronous diagnosis of 2 distinct types of PPB in a 2-year-old girl who presented with non-specific symptoms of an upper respiratory infection.

**Design/Method:** Case Report

**Results:** The patient's symptoms included one week of congestion, fatigue, cough, and sore throat followed by 1 day of fever. Her dyspnea and diminished right lung sounds prompted a chest x-ray which revealed a rounded right-sided pulmonary mass and possible pneumothorax. Chest CT scan confirmed a large heterogeneously solid and cystic mass of the right lower lobe and two small cysts in the right upper lobe. The patient underwent right lower lobectomy and wedge excision of the cysts. After pathology review, the final diagnosis was Type II PPB in the lower lobe and Type Ir PPB in both cystic lesions of the upper lobe. DICER testing was negative for a germline mutation but a mosaic mutation could not be excluded. The patient is being treated with 36 weeks of chemotherapy, including vincristine, ifosfamide, dactinomycin, and doxorubicin (IVADO). She remains in first remission.

**Conclusion:** Patients with Type Ir PPB are typically thought to have a lower risk of progressive or recurrent disease. This is the first individually reported example of synchronous Type Ir and Type II PPBs. There was an absence of DICER1 mutation which is unusual since most children with multiple PPBs, either synchronous or metachronous, have a germ line mutation.

Poster # 627

## ANTI-MALARIAL INDUCED METHEMOGLOBINEMIA IN A CASE OF G6PD DEFICIENCY WITH MALARIA

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**Background:** G6PD deficiency can increase MethHb levels. The same oxidative drugs that cause hemolysis in G6PD deficiency can also cause Methemoglobinemia. Recognition and management pose a unique challenge.

**Objectives:** To present a case of drug-induced methemoglobinemia with drug-induced hemolysis in a patient with G6PD deficiency

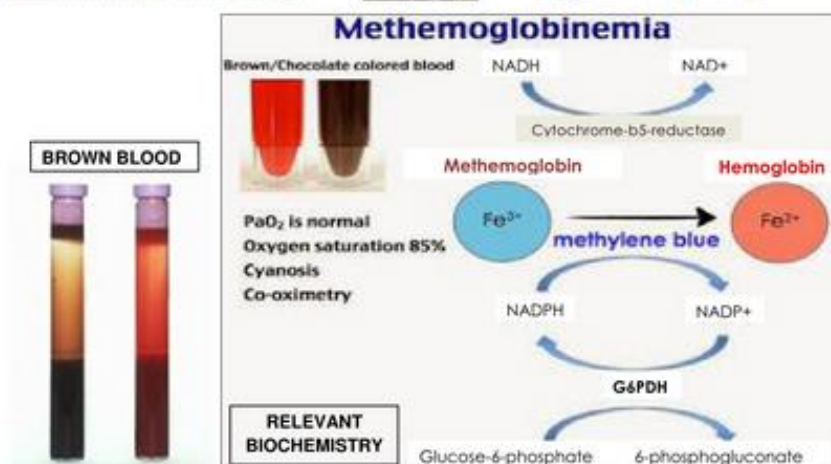
**Design/Method:** Case-study

**Results:** 7 year-old male with high grade fever, chills and rigors, diagnosed P.vivax malaria at a local clinic, received first 2 days of anti-malarials (chloroquine+primaquine). Fever subsided, but the mother noticed paleness, easy fatigability and jaundice. Confirmatory G6PD results sent earlier revealed moderate G6PD deficiency. Drug induced hemolysis in G6PD-deficiency was suspected. There was history of dark-colored urine. On examination, child was comfortable, with normal respiratory pattern. HR 100/min, RR 16/min, SaO<sub>2</sub> 84% on room air, BP 96/72 mmHg. There was moderate pallor, scleral icterus, cyanosis of the nail beds and mild splenomegaly. RS, CVS and CNS examination were normal. He was started on supplemental oxygen.

Reports revealed it was a case of Methemoglobinemia with G6PD deficiency with hemolysis. Methylene-blue was contraindicated (G6PD deficient + hemolysis). IV hydration, 100% O<sub>2</sub> and ascorbate were administered. MethHb and total Hb levels were monitored Q6H. Hb dropped to 6.8, necessitating packed cell transfusion. Post transfusion, MethHb levels reduced to 5% and the Hb stabilized at 9.6. Over 48 hours, MethHb normalized and Hb remained stable. Genetic testing is awaited.

**Conclusion:** Never administer anti-malarials without G6PD levels. Normal PaO<sub>2</sub> with reduced SaO<sub>2</sub> without respiratory distress, with brown-colored blood is characteristic of methemoglobinemia. Oxidative stress in G6PD deficiency can increase MethHb levels. IV Methylene-blue (treatment for Methemoglobinemia) is contraindicated in G6PD deficiency. Ascorbic acid is the alternative treatment, effective in mild cases. Child must be monitored for ongoing hemolysis and changes in mentation. Packed red cells must be transfused in case of drop in Hb and aids rapid recovery.

Parameter	0	6 hr	12 hr	18 hr	24 hr	30 hr	36 hr	48 hr
Hb (g/dL)	8.2	7.5	6.8	9.2	9.1	9.4	9.4	9.6
Haptoglobin	Reduced	-	-	-	Reduced	-	-	Normal
Meth Hb	18%	17%	14%	6%	6%	4%	2%	Undetectable
SaO <sub>2</sub> (Pulse oximetry)	84%	85%	84%	93%	93%	98%	98%	98%
SaO <sub>2</sub> (ABG)	95	95	94	98	97	96	97	99
PaO <sub>2</sub> (mmHg)	98	96	98	99	99	98	99	99
Urine color	Brown	Brown	Brown	Dark	Dark	Normal	Normal	Normal
S. Creatinine	1.1	-	1.1	-	0.9	-	-	0.8
Mentation	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Intervention	100% O <sub>2</sub> , Hydration, Ascorbate	100% O <sub>2</sub> , Hydration, Ascorbate	100% O <sub>2</sub> , Hydration, Ascorbate	100% O <sub>2</sub> , Hydration, Ascorbate	100% O <sub>2</sub> , Hydration, Ascorbate	100% O <sub>2</sub> , Hydration, Ascorbate	Ascorbate	Ascorbate



**DICER-1 SHOWING ITS UGLY FACE AGAIN. A NOVEL DICER1 MUTATION IDENTIFIED IN A FEMALE WITH AN UNDIFFERENTIATED RENAL SARCOMA WITH CYSTIC NEPHROMA BACKGROUND**

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**Background:** Germ-line mutations in the micro-ribonucleic acid processing gene DICER1 have been shown to predispose to a subset of benign tumors susceptible to malignant transformation, including cystic nephroma and pleuropulmonary blastoma. Many have viewed cystic nephroma as the benign end of the pathologic spectrum with cystic partially differentiated nephroblastoma and Wilms tumor. It has been recently argued that DICER1 mutations are the major genetic event in the development of cystic nephroma and that like pleuropulmonary blastoma in the lung, these tumors represents a spectrum of abnormal renal organogenesis with risk for malignant transformation.

**Objectives:** We present a case report of a 14 month female with enlarging abdominal mass found to have a DICER1 renal sarcoma with a novel loss of function mutation. To better understand the disease and to develop appropriate treatment guidelines and education and screening guidelines for individuals and families, we need to continue to explore and elucidate this disease process.

**Design/Method:** Despite limited reports of DICER1 renal tumors, a DICER1 renal sarcoma was suspected in a 14 month old Caucasian female who presented with a 1 week history of enlarging abdominal mass. Family history included a paternal uncle with multiple thyroid cysts and maternal aunt with ovarian cysts. Genetic analysis was performed on the tumor. Germline mutation testing is pending in the patient and her family members.

**Results:** The tumor had two distinct components with distinct genetic aberrations identified. The tumor had biallelic DICER1 mutations with a previously reported hotspot mutation, p.Glu1813Gly, in addition to a novel loss of function mutation, c.2651-2A>G. Genetic analysis revealed BRAF V600E mutation and gain of 17q in both components. The anaplastic components revealed a gain of 6q and loss of 9q that were not observed in the cystic nephroma components.

**Conclusion:** This is the fifth case report of a renal sarcoma associated with a cystic nephroma. Genetic analysis suggests that DICER1 mutations are the major genetic event in cystic nephroma formation but additional hits are needed to result in malignant transformation into renal sarcoma. The risk for transformation, responsiveness to chemotherapy and ultimate outcomes are all unknown but need to be further elucidated.

**IDIOPATHIC HYPEREOSINOPHILIC SYNDROME WITH CARDIAC THROMBUS IN A FOUR YEAR OLD GIRL, A CASE REPORT AND REVIEW OF THE LITERATURE**

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**Background:** Hypereosinophilic syndrome (HES) is defined as persistent hypereosinophilia (>1500 cells/ ml) for more than one month along with evidence of end organ damage. Idiopathic HES (IHES) is defined as HES without an underlying cause. Idiopathic HES is rare in children and has a male predominance.

**Objectives:** Describe a rare case of IHES, negative for *FIP1L1-PDGFR*A mutation causing cardiac thrombus in a young female child.

**Design/Method:** Single case report with review of literature.

**Results:** A four-year-old previously healthy girl presented with ten days of persistent fever, respiratory distress and left knee arthralgia. Clinical exam was remarkable for tachypnea and left knee joint tenderness without swelling or overlying erythema. A complete blood count (CBC) was significant for an elevated white blood cell count (39,000/ $\mu$ l) with 39% eosinophils (absolute eosinophil count 15,210/ $\mu$ l). Serum immunoglobulins including IgE levels were normal. Investigations for parasitic, fungal and mycobacterial infections were negative. Rheumatological studies were also unremarkable. A normal bone marrow aspirate and biopsy and a negative CAT scan of chest, abdomen and pelvis ruled out an underlying malignancy. During her hospitalization she developed junctional arrhythmia. Echocardiogram showed an echodense right ventricular mass, which on cardiac MRI was consistent with a thrombus at apex of the right ventricle. Anticoagulation with unfractionated heparin was started. FISH analysis for Fip1-like1-platelet derived growth factor receptor alpha (*FIP1L1-PDGFR*A) gene mutation was negative. A diagnosis of idiopathic HES was thus made and treatment with steroids was initiated. The patient responded both clinically and hematologically. Eighteen months after presentation, there is continued resolution of the thrombus but she had a relapse with hyperosinophilia and left knee pain. A repeat bone marrow showed no evidence of malignancy. Remission was again achieved with steroids with a plan to ultimately transition her to Hydroxyurea.

**Conclusion:** Idiopathic HES is rarely reported in children and has a male predominance. Only a few cases have been reported in young girls like our patient. Furthermore, cardiac involvement is mostly reported in boys with myeloproliferative variant, particularly with *FIP1L1-PDGFR*A mutation. Interestingly our patient developed cardiac symptoms but had none of the above-mentioned risk factors, making it a very unusual presentation.

Poster # 630

## **RAPID AND COMPLETE RESPONSE OF INFLAMMATORY MYOFIBROBLASTIC TUMOR OF BLADDER TO CELECOXIB**

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**Background:** Inflammatory myofibroblastic tumors (IMT) are tumors of borderline malignancy in children and young adults that most commonly affects the lungs, mesentery, omentum and very rarely the genitourinary tract. Surgical excision, the treatment of choice, is frequently not feasible in bladder tumors due to the excessive morbidity associated with resection of large tumors and tumors in close proximity to the bladder neck. Chemotherapy, radiation, steroids and non-steroidal anti-inflammatory drugs have been used to treat bladder IMTs with incomplete responses.

**Objectives:** We report a case of an unresectable bladder IMT that was successfully treated with celecoxib.

**Design/Method:** A 5-year-old male presented with persistent dysuria, lower abdominal pain and

low grade-fevers. He was treated empirically for a urinary tract infection but the abdominal pain persisted. A pelvic ultrasound showed a cystic mass on the dome of the bladder. Follow-up CT scan confirmed a left supero-anterior bladder dome mass measuring 5.3 cm x 4.8 cm x 3.0 cm with a cavitary fluid component measuring 1.2 cm x 1.3 cm x 1.6 cm. He had exploratory laparotomy but the mass was not amenable to primary excision. Biopsy was performed which confirmed the diagnosis of IMT. FISH for anaplastic lymphoma kinase (ALK) rearrangement was negative. Further metastatic evaluation with CT scan of the chest was negative. He was treated with celecoxib 250 mg/m<sup>2</sup> by mouth twice daily for 3 months.

**Results:** Abdominal pain and dysuria resolved within two weeks of initiation of celecoxib. CT scan of the pelvis performed 3 months later showed complete resolution of the tumor. Celecoxib was discontinued and he remains tumor-free for the past 20 months since discontinuation of celecoxib.

**Conclusion:** This case shows one of bladder IMT that completely responded to celecoxib monotherapy without a need for surgical intervention. Celecoxib may be a safe treatment option for patients with unresectable bladder IMT.

Poster # 631

## **SPONTANEOUS SPLENIC RUPTURE IN A NEONATE WITH SEVERE HEMOPHILIA A AND HEMOLYTIC DISEASE OF THE NEWBORN**

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**Background:** Neonatal splenic rupture occurs most commonly in the setting of a traumatic delivery. Spontaneous splenic rupture is extremely rare, usually occurring secondary to an underlying coagulation disorder or splenic abnormalities.

**Objectives:** To present a rare presentation of a neonate with splenic rupture who was successfully managed medically with preservation of his spleen, despite having both hemolytic disease of newborn (HDN) and severe hemophilia A.

**Design/Method:** History: An 11 day old term male who presented with abdominal distension, poor feeding and worsening jaundice. He had history of HDN with anti-A antibodies that required two days of phototherapy, one dose of IVIG and one packed red blood cell (pRBC) transfusion. Physical Exam: He had tachycardia, jaundice, and abdominal distension with palpable left flank mass. Workup: Lab work revealed hemoglobin 8.1 g/dl and total and direct bilirubin 15.6 and 0.4 mg/dl, respectively. Abdominal ultrasound showed an avascular heterogeneous left upper quadrant mass, confirmed by CT to be an active perisplenic hematoma. Subsequent coagulation studies showed a normal prothrombin time, prolonged activated partial thromboplastin time 76.5 sec and coagulation Factor VIII (FVIII) activity <1%. Clinical course: He received two pRBC transfusions and was started on recombinant FVIII infusions as 50 units/kg/dose every 8-hours to maintain trough FVIII activity near 100%. After 72 hours, infusions were gradually spaced and he continued to improve. One week after presentation, he was discharged home to complete a short course of recombinant FVIII infusions, but was then transitioned to an on-demand regimen.

**Results:** The classic triad of anemia, abdominal distension and shock are late findings in splenic rupture, making a prompt diagnosis of the utmost importance. Management ranges from conservative treatment to emergent splenectomy. With high index of suspicion and timely initiation of PRBCs transfusions and recombinant FVIII, this patient showed significant

improvement without the need for surgical intervention.

**Conclusion:** In newborns with splenic rupture, the possibility of concurrent causes should be ruled out, such as coagulation disorders and HDN causing splenic abnormalities. In newborns with hemophilia or other bleeding disorders, appropriate replacement of exogenous factor concentrates could prevent emergency splenectomy, which has not yet been reported.

Poster # 632

## **LATE METASTATIC RETINOBLASTOMA PRESENTING WITH SPINAL CORD COMPRESSION**

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**Background:** Metastatic retinoblastoma typically occurs within the first year after diagnosis. Spinal cord compression is a rare presentation of metastatic disease. Special consideration of treatment options is necessary due to the increased risk of second malignancies.

**Objectives:** Describe a case of late metastatic retinoblastoma presenting with spinal cord compression.

**Design/Method:** Case Report

**Results:** A 7-year-old male with history of bilateral retinoblastoma (RBL) presented with two-months of neck pain and right upper extremity weakness. Family history was notable for RBL in his father, paternal grandmother, paternal uncle and 3 sisters. He was diagnosed with RBL of the right eye at birth and RBL of the left eye six months later. Therapy included 4 cycles of carboplatin, 3 cycles of intra-arterial chemotherapy and plaque brachytherapy to the right eye, right eye enucleation, and laser therapy to the left eye. Initial work-up revealed moderate thrombocytopenia with a sclerotic lesion of the C6 vertebral body on X-ray. MRI revealed a 7cm x 5.6cm x 4.4cm epidural mass at the C5-C6 vertebral bodies with cord compression and an enhancing lesion in the T9 vertebral body. He received decadron and underwent biopsy of the cervical mass with bilateral bone marrow biopsies. Initial pathology showed diffuse small round blue cells in the lesion and marrow. Due to symptomatic cord compression, he was started emergently on cyclophosphamide, topotecan, and vincristine, a low-toxicity regimen with broad activity against small round blue cells. Final pathology of the cervical mass showed small round blue cell cells (synaptophysin positive, negative for CD45, CD99, AE1/3, myogenin, desmin, MSA, S100, NF, CD57, and MPO) consistent with metastatic RBL. Bilateral marrow results showed similar features with complex cytogenetics. He began induction therapy per ARET0321 with cisplatin, cyclophosphamide, and vincristine with a CR in his marrow and epidural mass after the first cycle. He is status-post 3 cycles of induction chemotherapy and preparing for consolidation with high dose chemotherapy with autologous stem cell rescue.

**Conclusion:** Late metastatic retinoblastoma, particularly with presentation as spinal cord compression, remains a rare occurrence. Avoidance of radiation therapy is critical in these patients due to their increased risk of secondary malignancy.

Poster # 633

## **BILATERAL NON-PROLIFERATIVE SICKLE CELL RETINOPATHY (NPSR) CAUSING PROFOUND VISION LOSS IN AN 8 YEAR OLD CHILD WITH SICKLE CELL DISEASE (SCD)**



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**Background:** Patients with SCD, especially sickle C disease (SCD-HbSC), are known to be especially prone to develop sickle retinopathy in middle age and later. Hence, yearly retinal examination is recommended for all patients with SCD after the age of 10 years for its early identification and treatment. Though sickle retinopathy is not uncommon in teenage years and later, it is extremely rare before 10 years of age. We would like to report a case of bilateral NPSR causing severe vision loss in an 8 year old child that was corrected and stabilized by monthly red cell transfusions (pRBCs).

**Objective:** We aim to present a previously never reported occurrence of bilateral NPSR in an eight year old with SCD which caused significant vision loss in the child.

**Design/Method:** On a regular clinic visit an eight year old boy with SCD-HbSS was discovered having decreased vision of unknown duration in right eye in spite having received 24 pRBCs over 3 years for other SCD complications until 5 months before this visit.

**Results:** Vision on initial presentation at Ophthalmologist's office was 20/400 and 20/20 in right and left eye respectively. Retinal examination showed salmon patch in macula of the right eye (intra-retinal hemorrhage - NPSR) while left eye showed NPSR with areas of intra-retinal hemorrhage in the peripheral retina. Fluorescein angiography showed good retinal perfusion in both eyes. Monthly simple transfusions were instituted promptly and continued indefinitely. His vision improved to 20/80 in right eye within 4 months and has remained stable subsequently after receiving 43 pRBCs over 3 years. Concurrently, intra-retinal hemorrhage in macula subsided leaving retinal pigmentary changes. He has had numerous sickle related co-morbidities including Acute Chest Syndrome, Moyamoya Syndrome, Transfusion Induced Iron Overload, Cholecystitis, Hyposthenuria resulting in nocturnal enuresis and behavioral issues at school.

**Conclusion:** Early institution of yearly retinal examinations, beginning at 5 years of age, will pick up early retinal changes in patients with SCD and help prevent permanent eye damage, especially for those at high risk. Where ophthalmologists are unavailable, good vision screening may help young children in early diagnosis of cases with macular involvement.

Poster # 634

## **A NON-GERMINONATOUS GERM CELL TUMOR IN A 15 YEAR OLD WITH SICKLE CELL DISEASE**

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**Background:** Primary central nervous system (CNS) germ cell tumors (GCTs) account for 0.5% of all primary brain tumors, with approximately 90% of the cases occurring before age 20 years.

**Objectives:** We discuss the case of a teenager with sickle cell disease (Hemoglobin SS phenotype) with development of significant neurologic complications who presented with non-germinomatous germ cell tumor.

**Design/Method:** Case Report

**Results:** A 15 year old male with sickle cell disease (Hemoglobin SS phenotype) consulted his physician due to worsening ataxia, urinary incontinence and facial asymmetry. Past medical

history was significant for a cerebrovascular accident in the left internal capsule of the basal ganglia two years prior and also a spontaneous right femoral vein thrombus. Physical examination was notable for right facial droop, 4/5 right-sided weakness in both the upper extremity and lower extremities, positive Babinski of the right foot and decreased sensation in the right hand and right lower extremity. Head MRI was concerning for tumor in the basal ganglia region. Intracranial biopsy was attempted twice with eventual diagnosis of mixed non-germinomatous germ cell tumor. Laboratory findings were significant for elevated beta-hCG at 22.7 mIU/mL in the serum and 51.8 mIU/mL in the cerebrospinal fluid. He was started on treatment as per Children's Oncology Group protocol ACNS0122 with chemotherapy and radiation therapy. He has been in clinical, radiologic and chemical remission since completion of radiation and chemotherapy.

**Conclusion:** This case report is the first to describe concurrent non-germinomatous germ cell tumor and sickle cell disease.

Poster # 635

## **SEVERE COMBINED IMMUNODEFICIENCY (SCID) PRESENTING AS NEONATAL APLASTIC ANEMIA**

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**Background:** Neonatal aplastic anemia is uncommon and has a broad differential including infections, drugs/toxins, myelodysplastic syndromes, inherited marrow failure syndromes and immune disorders. We report the unique case of severe combined immunodeficiency (SCID) presenting with neonatal aplastic anemia. Our patient met clinical criteria for SCID which remains genetically undefined. This report highlights the importance of considering SCID early in the evaluation of neonatal aplastic anemia prior to the development of infectious complications.

**Objectives:** To extend the differential diagnosis of neonatal aplastic anemia.

**Design/Method:** A single case-report of neonatal aplastic anemia as the presenting symptom in an infant with SCID.

**Results:** A full term female presented with extensive bruising at birth. Initial CBC demonstrated thrombocytopenia, anemia and lymphopenia. An extensive evaluation for infection was negative and she was treated empirically with broad spectrum antibiotics. She developed progressive anemia, persistent thrombocytopenia and severe neutropenia within the first two weeks of life necessitating transfusion support. A bone marrow biopsy revealed marked aplasia (10% cellularity) without dysplasia or maturation arrest. Cytogenetics and FISH were not performed due to insufficient sample volume. An evaluation for inherited bone marrow failure syndromes was negative. At three months of age she was admitted for fever with neutropenia. An immunologic evaluation was sent. Prior to these results, she developed respiratory distress and underwent bronchoscopy that demonstrated *Pneumocystis jiroveci*. She was started on Bactrim and G-CSF therapy. Her course was further complicated by pulmonary aspergillosis requiring granulocyte transfusions and IVIG. Immunologic evaluation revealed a genetically undefined T positive, B negative, NK negative leaky SCID. She underwent a human leukocyte antigen (HLA) matched unrelated donor bone marrow transplant following nonmyeloablative conditioning. She rejected the graft and underwent a second HLA matched unrelated donor peripheral blood stem

cell (PBSC) HCT following reduced-intensity conditioning. She is currently alive and well with full donor engraftment one year following transplant.

**Conclusion:** This case highlights the importance of early consideration of SCID in the evaluation of neonatal aplastic anemia prior to the development of infectious complications. Diagnosis of SCID is critical for timely initiation of antimicrobial prophylaxis and curative therapy with bone marrow transplant.

Poster # 636

## OVARY PRESERVATION IN THE TREATMENT OF CHILDHOOD MEIGS SYNDROME: A CASE REPORT AND LITERATURE REVIEW

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**Background:** Meigs syndrome, the combination of benign ovarian tumor, ascites and pleural effusion, is present in a small percentage of ovarian fibromas and infrequently reported in children. When accompanied by an elevated CA-125 level, the findings raise suspicion for malignancy often prompting aggressive surgical intervention. We present a case and review of childhood Meigs syndrome, with particular emphasis on management that spares the ovary.

**Objectives:** To describe a case of Meigs syndrome in an adolescent, review the literature and previously reported cases, and increase provider awareness of the condition to minimize unnecessary oophorectomy.

**Design/Method:** PubMed and Medline searches were performed for cases involving benign ovarian tumors associated with pleural effusion and ascites in patients under age 21. Cysts, ovarian torsion, and edematous ovaries were excluded.

**Results:** Nine cases of childhood Meigs syndrome were identified (Table 1). Salpingo-oophorectomy was performed in all but one, in which the ovary was spared. There were no cases that recurred or progressed to malignancy.

**Conclusion:** Ovarian tumors associated with abdominal ascites, pleural effusion, and elevated CA-125 raise concern for malignancy. High CA-125 is typically associated with advanced epithelial ovarian cancer in adults. However, the constellation of findings in children most commonly indicates a benign ovarian fibroma. Our report and review highlights the importance of presurgical identification of Meigs syndrome so that every attempt can be made to avoid oophorectomy and preserve optimal fertility when feasible.

**Table 1: Reported Cases of Meigs Syndrome in Patients Under Age 21**

	Author	Journal	Year	Age (years)	Histopathology	Size (cm)	CA-125 Level (U/ml)	Ascites (ml)	Management	Outcome/Follow up
1	Slovin and Nealon	Delaware Medical Journal	1951	20	Fibroma	15.5	NA	10000	Lt. salpingo-oophorectomy	Resolution by post-op day 8. No long term follow up reported.
2	Latta and Lee	J. Adoles Healthcare	1981	20	Fibroma and dermoid cyst components	14	NA	700	Rt. salpingo-oophorectomy	Radiologic clearing of pleural effusion within 1 week post-op.
3	Chan et al	British J. of Radiology	2000	13	Fibroma	20x19x10	970	2000	Lt. salpingo-oophorectomy and omentectomy	Complete resolution of pleural effusion within 5 days. No long term follow up reported.
4	Bildirici et al	Eur. J. Gynaec. Oncol.	2004	17	Sclerosing stromal tumor	20.5x10.9	193	400	Rt. salpingo-oophorectomy	No recurrence at 2 years.
5	Liou et al	Taiwanese J. of Obstetrics & Gynecology	2010	18	Sclerosing stromal tumor	14.5x13x9.5	4208	9000	Rt. salpingo-oophorectomy	Return to baseline of CA-125 and ascites. No long term follow up reported.
6	Sugiyama et al	J. Ped Surg	2011	12	Fibroma	11x9.5x9	1170	6700	Lt. ovarian tumor enucleation with sparing of the ovary	No recurrence at 1 year post-op.
7	Monteiro et al	J. Ped Adolesc Gyn	2012	13	Mitotically active ovarian fibroma	14	453	3000	Rt. salpingo-oophorectomy, infracolic omentectomy and peritoneal biopsy	No recurrence at 3 years post-op with normal tumor markers and ultrasound.
8	Gomes Sobrinho et al	Rev. Bras. Gyn Onc	2013	17	Sclerosing stromal tumor	221.3 cm <sup>3</sup>	625	20000	Rt. salpingo-oophorectomy	No recurrence at 7 years post-op.
9	Current report		2014	13	Fibroma	17x19	560	1000	salpingo-oophorectomy	No recurrence at 1 year post-op.

## **PULMONARY COMPLICATIONS AND FUNCTIONAL ASSESSMENT OF LUNG FIBROBLASTS IN A PEDIATRIC PATIENT WITH DYSKERATOSIS CONGENITA**

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**Background:** Dyskeratosis congenita (DC) is a congenital bone marrow failure disorder characterized by abnormal skin pigmentation, nail dystrophy, oral leukoplakia and cancer predisposition. DC is caused by defective telomere maintenance, and several studies have shown aberrant DNA damage responses in DC cells. Pulmonary fibrosis is considered a late finding in DC. However, pulmonary complications are frequent in DC patients undergoing hematopoietic stem cell transplant (HSCT).

**Objectives:** We describe a 17 year old male with x-linked DC with pulmonary complications pre and post allogeneic HSCT, and additionally characterize *in vitro* function of lung fibroblasts from this patient.

**Design/Method:** A case report of a patient with X-linked DC who underwent HSCT. Additionally, pulmonary tissue collected post-mortem was used to generate lung fibroblasts. Control, DC, and cystic fibrosis (CF) lung fibroblasts were assessed *in vitro* for proliferative capacity. Additionally reactive oxidative species (ROS) were quantitated using specific fluorochromes and flow cytometry under normoxic and hypoxic conditions.

**Results:** Our patient presented at 1 year of age with mild anemia and subsequently developed classic mucocutaneous findings consistent with DC. At age 10, androgen therapy was initiated for progressive pancytopenia. He had occasional dyspnea but pulmonary function testing was normal. By age 16, a progressive decline in diffusion capacity was noted. Radiographs revealed multiple apical blebs. Due to progressive marrow aplasia, he underwent a reduced intensity matched sibling allogeneic HSCT. His post-transplant course was complicated by CMV reactivation, pulmonary embolism, oxygen dependency and progressive extension of pneumatoceles, eventually leading to cardiorespiratory failure. Post mortem exam of the lungs revealed extensive pulmonary blebs with histologic evidence of fibrosis. Cultured DC lung fibroblasts demonstrated marked growth impairment and elevated reactive oxidative species (ROS) compared to control and CF lung fibroblasts. Importantly, DC fibroblast proliferation normalized under hypoxic conditions.

**Conclusion:** Pulmonary dysfunction is seen in a subset of DC patients and is common post HSCT. Our findings of impaired pulmonary fibroblast proliferation and elevated ROS suggest an intolerance of DC lung tissue to oxidative stress and impaired DNA damage repair mechanisms. These observations support the use of non-ablative HSCT conditioning regimens in DC.

## **CONGENITAL TRANSFORMATION OF SACROCOCCYGEAL TERATOMA TO MALIGNANT CENTRAL PRIMITIVE NEUROECTODERMAL TUMOR: A CASE REPORT**

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**Background:** The incidence of congenital tumors is increasing with extracranial teratomas being most common. This case report discusses a unique malignant transformation of a common benign tumor, which has not previously been discussed in the literature.

**Objectives:** Discuss management of a sacrococcygeal teratoma that underwent malignant transformation in utero to a central primitive neuroectodermal tumor.

**Design/Method:** Case Report

**Results:** Infant was born full-term after a pregnancy complicated by advanced maternal age and abnormal quad screen. Mother received adequate prenatal care and was on vitamins. Delivery was uncomplicated. After birth, a 2-3 cm sacrococcygeal mass was noted on the baby. She underwent gross total resection with potentially negative surgical margins on day of life two as the mass was assumed to be a benign sacrococcygeal teratoma. However, pathology was reviewed by specialists and consensus determined that the mass contained teratomatous elements as well as a transformation of ninety-five percent of the tumor to a high-grade malignant component that resembled central primitive neuroectodermal tumor (cPNET). Initial literature review showed no case reports of malignant transformation of sacrococcygeal teratomas, although there were reports of malignant transformation of testicular and ovarian teratomas. Review of the literature suggested most successful results occurred in patients treated with PNET-based chemotherapy. Metastatic evaluation was completed and negative in the case of this infant. Consequently, plan was made for observation only without using chemotherapy given the lack of metastatic disease and to avoid developmental compromise with chemotherapy. This decision was made in part on review of cases where complete surgical resection in patients with localized disease was curative in adult males with testicular teratomas with cPNET malignant transformation. Our patient was examined monthly and had repeat imaging every three months. The family was adherent with the monitoring. One year later, the baby has had no findings of recurrence, as all her physical exams and MRIs have been negative. Her only complication from her mass and surgery is constipation. She is developing and growing normally.

**Conclusion:** Despite the degree of malignant transformation, it appears conservative management was an appropriate choice in the setting of surgical resection and no metastatic disease. Continued surveillance though remains necessary.

Poster # 639

## **SUCCESSFUL CATHETER-DIRECTED THROMBOLYSIS IN A PEDIATRIC PATIENT WITH KNOWN PROTEIN S DEFICIENCY**

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**Background:** Catheter-directed thrombolysis (CDT) has been shown to be effective and safe for severe pulmonary embolism (PE) in adults but its application in the pediatric population has been limited. CDT accelerates fibrinolysis of a thrombus by local administration of tissue plasminogen activator (tPA) while reducing systemic bleeding risk. This can be achieved with lower dosing and shorter treatment times in CDT as compared to systemic tPA administration

and heparinization. Poor outcomes have been shown in patients with PE and right ventricular (RV) dysfunction leading to potential RV failure and cardiogenic shock.

**Objectives:** Case report of a fifteen-year-old female with known Protein S deficiency, presenting with large bilateral pulmonary emboli, successfully treated with CDT by interventional radiology (IR).

**Design/Method:** Case report

**Results:** A fifteen-year-old female with Protein S deficiency and developmental delay without prior medical history presented with two syncopal episodes and shortness of breath. A CT chest showed filling defects of the right main pulmonary artery and left pulmonary artery, suggestive of bilateral pulmonary emboli, originating from a 6 cm thrombus extending from the left external iliac vein into inferior vena cava. Patient was initially started on enoxaparin, followed by transfemoral placement of catheters into each pulmonary artery for bolus tPA administration *in situ* via CDT and consequently continuous tPA infusion (1mg/hr for 20 hours). No systemic heparinization was added. There were no major bleeding issues and the procedure was hemodynamically well tolerated. Angiography showed complete resolution of both large PEs and widening of the intraluminal blood flow in the vena cava after CDT. Enoxaparin was restarted post-intervention. No oral anticoagulation was initiated due to the patient's persistent refusal and incapacity to take oral medication.

**Conclusion:** Catheter directed thrombolysis with tPA is a safe and successful alternative to treat pediatric patients with severe pulmonary emboli. Fast resolution of the clot reduces the morbidity, while intensive monitoring is warranted for bleeding assessment.

**References:**

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Poster # 640

## **A REPORT OF A CHILD WITH A PITUITARY BLASTOMA AND DICER1 SYNDROME**

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**Background:** Pituitary blastoma is an extremely rare brain tumor that typically presents in early childhood. This rare tumor has been found to be an important marker for the DICER1 syndrome or the familial pleuropulmonary blastoma syndrome, which is a cancer predisposing syndrome.

**Objectives:** To report a case of a child with a pituitary blastoma, suggestive of the DICER1 Syndrome, and discuss his approach to management.

**Design/Method:** We present a case of a 22 month old male who presented to the hospital with a several month history of intermittent headaches and vomiting. MRI of the brain showed a two centimeter expansile sellar- based lesion with cystic and solid components, thought initially to be a craniopharyngioma. A total resection of the pituitary mass was performed shortly thereafter. Pathology was pituitary blastoma. A reference lab reviewed the case and, suspecting DICER 1 syndrome, did sequencing which revealed a missense mutation (NM\_177438.2:c.5125G>A; p.Asp1709Asn) located at one of the five "hotspot" amino acids in the DICER1 RNase IIIb domain. This is a characteristic feature of DICER1 syndrome tumors. Further work up included CT of the chest which showed multiple small pulmonary blebs in the posterior peripheral region of the lungs.

**Results:** There is conflicting information on the need to treat children with gross total resections of pituitary blastomas. Since this child had a gross total resection, no further treatment was given. Subsequent MRIs, now over 4 months post-resection, have shown no evidence of recurrent disease. The patient is closely followed by endocrinology for panhypopituitarism and diabetes insipidus due to his tumor resection.

**Conclusion:** It is crucial to identify and report any cases of this very rare tumor. There is an International Pleuropulmonary Blastoma Registry that collects incidence and treatment information for these patients and families. Some patients with pituitary blastoma may survive without aggressive therapy.

Poster # 641

### **SEVERE ANEMIA SECONDARY TO CONCOMITANT OCCURRENCE OF STOMATOCYTOSIS AND HEREDITARY SPHEROCYTOSIS (HS) IN A PATIENT WITH PARENTERAL NUTRITION-ASSOCIATED LIVER DISEASE (PNALD)**

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**Background:** Homozygous or compound heterozygous mutations in the EPB42 gene which codes for protein 4.2, an integral component of red blood cell (RBC) cytoskeleton, have been shown to cause autosomal recessive HS.

**Objectives:** We describe a novel case of HS associated with a heterozygous mutation in the EPB42 gene (protein4.2) complicating anemia in an 18 month old European American male with short gut syndrome and PNALD who also had remarkable stomatocytosis on peripheral blood smear.

**Design/Method:** Case report. Osmotic fragility and ektacytometry was used to evaluate the phenotype of the patient's RBC cytoskeleton. Next-Generation Sequencing (NGS) was performed on DNA isolated from peripheral blood for 10 RBC membrane-related genes, by a CLIA-certified assay. All variants identified were confirmed by Sanger sequencing of the exon or intron involved.

**Results:** An 18 month old European American male with history of gastroschisis, jejunal atresia, short gut syndrome after bowel resection and PNALD was found to have anemia (hemoglobin of 8.8 g/dL, MCV 83.5 fL, MCHC 32.9 g/dL) with undetectable haptoglobin indicating hemolysis. Blood smear was significant for occasional spherocytes at six months of age and remarkable stomatocytosis. Eosin-5-maleimide screening test for hereditary spherocytosis was normal but osmotic fragility was increased. Ektacytometry confirmed increased osmotic fragility and decreased deformability of the erythrocytes compatible with HS. NGS of 10 genes associated with RBC membrane disorders showed a novel heterozygous mutation in EPB42 gene (c.826C>T; p.R276W). This mutation results in substitution of tryptophan for arginine at amino acid 276 in RBC membrane protein 4.2. Through the use of mutation prediction software, this amino acid substitution is predicted to be "deleterious" and "probably damaging".

**Conclusion:** We present a hitherto unreported case of mild hereditary spherocytosis in association with heterozygous mutation in EPB42 gene with concurrent occurrence of stomatocytosis probably secondary to PNALD causing altered consistency of the RBC lipid bilayer and decreased RBC survival. Although only a single EPB42 gene mutation was found it is likely that this patient may have a large deletion or other structural abnormality on his second

allele that is not detectable by NGS.

Poster # 642

## **A REPORT OF OSTEOSARCOMA IN A CHILD WITH TRISOMY 21**

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**Background:** The diagnosis of osteosarcoma is seen extremely rarely in trisomy 21 patients. There are currently no patients in the literature reporting on this association. Chemotherapy planning is especially important given that high dose methotrexate at  $12\text{g/m}^2$  has never been given to a child with trisomy 21. We also know that children with trisomy 21 do not tolerate high dose methotrexate well.

**Objectives:** To report a unusual case of osteosarcoma in a patient with trisomy 21 and discuss an appropriate treatment plan.

**Design/Method:** We present a case of a 13 year old female with a history of trisomy 21 and ventricular septal defect who presented to her primary care physician with a two day history of left ankle swelling. An x-ray was done that raised concern for a mass of the distal left tibia. Subsequently, an MRI was obtained and the result was consistent with a neoplastic lesion. A biopsy and culture of the lesion were performed that showed a high grade conventional osteosarcoma. A CT of the chest confirmed metastatic disease with multiple bilateral pulmonary nodules.

**Results:** After an extensive literature search and a discussion with many colleagues, a therapeutic plan using cisplatin, modified doxorubicin with dexrazoxane, and ifosfamide has been planned. Surgery will proceed after two initial rounds of chemotherapy. The patient tolerated her first cycle of cisplatin/doxorubicin well. Cisplatin was administered at 70% dosing; doxorubicin was administered at full dose but over 15 minutes instead of over 24 hours.

**Conclusion:** Children with trisomy 21 represent a high risk group when receiving chemotherapy; this is to our knowledge is the only case in the literature of a child with osteosarcoma and trisomy 21. We submit this as a report that may aid other physicians in planning therapy for similar patients in the future. A follow up will be published when the patient has completed therapy.

Poster # 643

## **DASATINIB INDUCED CHYLOTHORAX AND NEPHROTIC SYNDROME IN A PEDIATRIC PATIENT**

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**Background:** Dasatinib is a tyrosine kinase inhibitor (TKI) approved for the treatment of Philadelphia chromosome positive acute lymphoblastic leukemia (Ph+ALL). It is usually well tolerated, but can cause multiple side effects including extra-hematologic. Pleural effusions are the most common extra-hematological toxicity observed. Reported primarily in adults, studies have indicated that the likelihood of pleural effusion increases with twice daily dosing and



increasing age. Nephrotic syndrome is a rarely reported side effect particularly among pediatric patients. Here we describe a 14y/o male who developed nephrotic syndrome and bilateral chylothoraces after 10 months of Dasatinib treatment.

**Objectives:** Describe off-target kinase effects of Dasatinib in a pediatric patient, its management and effects on treatment.

**Design/Method:** Retrospective chart review

**Results:** 14-year-old male was diagnosed with Ph+ pre-B ALL and treated with Dasatinib, 60mg/m<sup>2</sup>/dose. 10 months after initiation, patient was admitted with salmonella sepsis, and noted to have anasarca, bilateral pleural and pericardial effusions. Patient also had proteinuria and albuminuria and was diagnosed with nephrotic syndrome. Patient was managed with fluid restriction, diuretics and albumin infusions. 5 weeks later, patient presented with productive cough and shortness of breath. On physical exam, patient was hypoxic with decreased breath sounds on lung bases. Chest x-ray revealed large bilateral pleural effusions. Pleural fluid analysis was consistent with chylothorax. Dasatinib was held during this time, and patient was placed on a low fat diet. Within 2 weeks, chylothorax resolved and the patient was restarted on Dasatinib 48mg/m<sup>2</sup>/day (20% reduction of original dose). Patient has tolerated this dose without recurrence of nephrotic syndrome or chylothorax and is currently in the continuation phase of treatment.

**Conclusion:** The exact pathogenesis of chylothorax and nephrotic syndrome secondary to Dasatinib is unknown. The simultaneous presence of both side effects suggests a possible common pathway. Off-target kinase inhibition of cytokines such as platelet-derived growth factor receptor (PDGFR)-beta may interfere with pericytes and mesangial cells affecting kidney function, in addition to interrupting lymphatic endothelial cell proliferation and migration in lymphangiogenesis. As in this patient, it is possible that lower doses of Dasatinib could still be effective in Ph+ALL treatment without undue interruption of critical endothelial function.

Poster # 644

## DIFFICULT DIAGNOSIS AND TREATMENT OF AN ADOLESCENT RENAL TUMOR

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**Background:** Approximately two-thirds of renal malignancies in adolescents (aged 15-19 years) are renal cell carcinomas (RCC). While treatment for localized RCC is more uniformed, there is no specific treatment for metastatic disease, and relies heavily on molecularly targeted therapy.

**Objectives:** We report an adolescent male with a right-sided renal mass that histologically resembled multiple tumor types. He was eventually diagnosed with a metastatic biphasic malignant neoplasm and encountered multiple adverse events from his medical treatment leading to treatment with a molecularly targeted agent.

**Design/Method:** Case Report

**Results:** A 16-year-old male presented with an abdominal mass (18.1 cm x 16.1 cm x 21.0 cm) extending from his right renal fossa. Laboratory data was unremarkable except for an elevated CA-125 and a biopsy suggested sarcomatous RCC. Radical resection revealed metastatic lesions to the colon, IVC, and lymph nodes. Gross pathology demonstrated several histological subtypes: synovial sarcoma, RCC, and Wilms' tumor. Immunohistochemistry staining was unremarkable and PCR analysis for synovial cell t(X;18) was negative. Initial treatment consisted of 6 courses of doxorubicin and gemcitabine. Due to concerns of lifetime anthracycline exposure, the treatment regimen was modified by incorporating docetaxel and

bevacizumab to the gemcitabine backbone. Unfortunately, the patient then developed nephrotic range proteinuria, grade 3 hypertension, and a kidney biopsy revealed thrombotic microangiopathy (TMA). Molecular sequencing revealed mutations in BRAF D594G and FAM123B genes. This BRAF mutation is a kinase impaired BRAF mutation leading to activation of the MEK-ERK pathway. Thus, the patient began treatment with trametinib, a MEK inhibitor. He is currently 12 months from diagnosis and showing no evidence of disease.

**Conclusion:** After pathologists at multiple institutions analyzed the tumor, it was agreed that the dominant cell type was synovial sarcoma; however, given the existence of histologic variation and the absence of an SYT translocation seen in >90% of synovial sarcomas, a specific diagnosis was impossible. Molecular studies revealed a targetable mutation leading to a specific treatment that has been better tolerated when compared to traditional chemotherapy. This joins an increasing amount of literature that highlights the benefit of molecular testing, especially in challenging cases.

Poster # 645

## **PEDIATRIC ACUTE PROMYELOCYTIC LEUKEMIA PRESENTING AS ISCHEMIC STROKE: A COAGULATION CONUNDRUM**

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**Background:** The most common cause of treatment failure in pediatric acute promyelocytic leukemia (APL) is severe hemorrhage. This is further complicated by concurrent thrombophilia. This dichotomous propensity for both bleeding and clotting introduces management challenges. Here we describe a novel presentation of pediatric APL as ischemic stroke, and therapeutic strategies aimed at achieving this critical balance.

**Objectives:** Discuss the mechanisms of simultaneous thrombophilia and severe bleeding in APL, and explore challenges of treatment.

**Design/Method:** A five year-old female presented to the ED with confusion, aphasia and right-sided facial weakness after trauma three days prior. She was able to follow commands, but developed right-sided hemiplegia with difficulty swallowing. Head MRI revealed left middle cerebral artery occlusion with infarction. She was transferred to the PICU and started on a heparin drip. Her CBC showed pancytopenia, coagulation labs were unremarkable. Blood smear revealed 15% blasts with Auer rods. Bone marrow biopsy diagnosed APL, with FISH positive t(15;17). Treatment with all-trans-retinoic acid (ATRA) was readily initiated. On hospital day seven she had up-going left-sided Babinski. Repeat MRI showed progression of her initial infarct and new midbrain hemorrhage. She stabilized with continued medical management. She completed induction with ATRA, idarubicin and arsenic trioxide (ATO), and was transferred to a rehabilitation facility on consolidation therapy.

**Results:** APL presenting as ischemic stroke, complicated by hemorrhage with ATRA, outlines the complexity of this disease. With dueling risks of bleeding and thrombosis, APL introduces a coagulation conundrum, often in the absence of standard coagulation lab abnormalities. These conflicting scenarios are a result of disseminated intravascular coagulation (DIC), fibrinolysis and proteolysis. Circulating procoagulants factors have been identified in APL, contributing to thrombophilia which may induce DIC. Leukemic promyelocyte expression of Annexin A2 promotes fibrinolysis. Granules seen in these same cells release proteases which degrade fibrinogen and procoagulant mediators. Treatment with ATRA further complicates these

conditions inducing hyperleukocytosis, which can exacerbate the coagulopathy.

**Conclusion:** This case demonstrates the vital importance of continuous clinical assessment guiding the management of pediatric APL in the context of severe, conflicting hazards of thrombophilia and catastrophic bleeding which may exist with normal coagulation labs.

Poster # 646

## **LONG-TERM USE OF SIROLIMUS IN PTEN HAMARTOMA TUMOR SYNDROME – CASE SERIES**

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**Background:** PTEN Hamartoma Tumor Syndrome (PHTS) is a rare cancer predisposition condition manifested in early adulthood with ovarian, endometrial or thyroid cancers. During childhood though, it may present with debilitating vascular anomalies and hamartomas causing severe chronic pain and limitation of range of motion.

**Objectives:** To study the efficiency of sirolimus (mTOR inhibitor) to 1. Control the growth of hamartomas and vascular anomalies and 2. Offer symptomatic relief for patients affected by PHTS.

**Design/Method:** Four patients (2 boys and 2 girls) with documented PTEN mutation, vascular anomalies, hamartomas and chronic pain/limitation of range of motion were consented to receive Sirolimus with a target trough level of 10-15ng/dl. All of them are still on treatment (3 months to 5 years intermittently). A retrospective chart review was performed and the results analyzed.

**Results:** All the patients (ages 6 to 11yo) at the start of therapy manifested response to sirolimus expressed as decreased pain due to the lesions (4/4) and decreased size of the lesions (3/4) over the first several months to 1 year of therapy. Three of the patients experienced relapse/flare of their disease (masses enlarged and/or became painful again) when Sirolimus was stopped for several months to a year. Currently all 4 remain on Sirolimus, with the expected changes in their serum lipid profiles, though none have required lipid lowering agents, or other interventions for toxicity.

**Conclusion:** 1. Sirolimus is a well-tolerated oral therapy without major side effects even during long-term use. 2. Sirolimus offers significantly improved quality of life with pain control and improvement of range of motion of the affected areas. 3. Sirolimus does not achieve dependence or tolerance over long-term use, and may be administered intermittently if needed. 4. Symptoms recur during interruption of therapy.

Poster # 647

## **POSTERIOR REVERSIBLE ENCEPHALOPATHY SYNDROME AND CEREBRAL SINUS THROMBOSIS IN A CASE OF PEDIATRIC B CELL ALL**

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**Background:** Despite our vastly more successful current treatments, pediatric patients with leukemia continue to suffer from numerous side effects of the disease and their treatments.

Posterior reversible encephalopathy syndrome (PRES) and cerebral sinus thrombosis are two such known complications. Thromboses result from the hypercoagulable state of malignancy and therapy, and PRES is thought to be treatment related toxicity.

**Objectives:** A case review of one patient whose disease was complicated by both of these conditions, calling into question our current understanding of the risk factors and pathophysiology of PRES. Our case is novel both for the fact that PRES developed before the initiation of therapy, and for the fact that both of these intracranial pathologies occurred in the same patient.

**Design/Method:** Single case review, performed at the Nemours/A.I.duPont Hospital For Children in Delaware.

**Results:** A previously healthy three year old presented with recurrent fevers and was diagnosed with ALL. At presentation, she had hypertension and an elevated lumbar puncture opening pressure, leading to a diagnosis of PRES, confirmed by MRI. After induction chemotherapy, a follow up MRI noted improvement of the PRES but also a new cerebral sinus thrombosis. Testing for secondary causes for PRES and genetic causes of hypercoagulability were all negative.

**Conclusion:** This case raises questions about our current understanding of the pathophysiology of PRES, suggesting that PRES may precede initiation of induction chemotherapy in children with ALL and may be a predisposing factor for cerebral sinus thrombosis. PRES is usually thought to be a complication of treatment. Current thinking suggests chemotherapy and steroids are responsible for PRES in ALL. Unique to this patient, however, PRES occurred before treatment started, suggesting that ALL itself may be a risk factor for PRES. The implication here is that clinicians should be alert to the possibility of PRES in a hypertensive oncology patient, even before therapy begins, and close attention should be paid to the lumbar puncture opening pressure and other signs of increased intracranial pressure. Our case also raises the possibility of an association between PRES and cerebral sinus thrombosis.

Poster # 648

## **EPITHELIOID SARCOMA WITH PLACENTAL METASTASIS IN A PREGNANT ADOLESCENT FEMALE**

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**Background:** Epithelioid sarcoma (ES) is an uncommon subtype of soft tissue sarcoma that occurs at a median age of 23-40 years at diagnosis. Malignancy in pregnancy is also regarded as an infrequent phenomenon, with an occurrence estimated at about one per 1,000 births. There are few published studies concerning effective therapy in ES and even less reports in the literature concerning sarcomatous spread into the placenta.

**Objectives:** To highlight the significance of intervillous involvement and possible implications for cancer surveillance in the infant.

**Design/Method:** Case report

**Results:** A 17 year old G1P0 female presented at 31 weeks gestation for evaluation of an ulcerated wound involving her right fifth digit. The lesion initially appeared as a small wart at the age of 10 but started ulcerating over the past year. Two months prior to evaluation, she developed large masses over her right chest wall and axilla. Biopsy confirmed ES, and staging studies revealed widely metastatic disease. Given the significant morbidity associated with high

dose ifosfamide and doxorubicin therapy in a gravid patient with minimal curative benefit, decision was made to defer therapy until after the delivery of the infant. She was induced at 34 weeks gestation and therapy initiated, but the patient clinically deteriorated shortly after and died. Placental pathology demonstrated intervillous invasion of ES. As most cases of infant disease occurred within the first 12 months, decision was made for whole body surveillance MRI every 3 months for the first year of life. At 10 months of age, the infant remains free of disease.

**Conclusion:** There are no guidelines concerning appropriate surveillance of infants born to mothers with sarcoma and placental metastases. Reports of fetal spread have proposed that intervillous invasions may place infants at considerable risk for disease. This is the first reported case of epithelioid sarcomatous placental invasion in a pediatric patient. With many non-teratogenic chemotherapeutic agents available, the option of therapy should be offered to a pregnant patient to possibly decrease the risk of metastatic spread to the placenta and, therefore, decrease the risk of disease in the infant.

Poster # 649

## **EBV LYMPHOPROLIFERATIVE DISEASE OF THE CNS IN A CHILD WITH LOW RISK PRE-B CELL ACUTE LYMPHOBLASTIC LEUKEMIA**

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**Background:** EBV-associated lymphoproliferative disease is a known complication of severely immunocompromised patients following allogeneic bone marrow or solid organ transplant. It has rarely been reported in patients with pre-B acute lymphoblastic leukemia (ALL), especially in patients considered low risk based on MRD and cytogenetics.

**Objectives:** To present the case of a 3-year-old female with low risk pre-B ALL diagnosed with EBV lymphoproliferative disease of the CNS during maintenance therapy

**Design/Method:** Case Report

**Results:** A 3-year-old female with low risk pre-B cell ALL in maintenance therapy presented with a febrile illness, 'noisy breathing' and a hoarse voice after several months of recurrent respiratory infections and fevers, in the setting of neutropenia. She was found to have adenoiditis with right vocal cord edema and tissue swelling. Pharyngeal culture grew *Candida Albicans*, with a subsequent CT of the chest revealing multiple nodules concerning for yeast. High fevers continued despite appropriate antibiotic and antifungal therapy. On hospital day 10, the patient developed acute onset of focal neurologic findings including head bobbing, eye rolling and left lip droop. MRI of the brain showed a focal enhancing lesion at the right posterior-parietal area and multiple edematous, non-enhancing areas. CSF and blood analysis were positive for EBV infection. Brain biopsy was positive for EBV (EBER positive) and consistent with B-cell lymphoproliferative disease. Adenoid and vocal cord tissue were also positive for EBV. The patient was removed from all immunosuppressive therapy and is being treated with daily amphotericin for CNS and posterior pharynx candidiasis. Neurologic symptoms have started to improve.

**Conclusion:** EBV lymphoproliferative disease can occur in any immunosuppressed patient, including pediatric patients with low risk pre-B cell ALL. Although rare in this population, one should maintain a high suspicion for lymphoproliferative disease in all patients receiving chemotherapy or other immunosuppressive treatment due to the induced T-cell defect.

## A RARE PRESENTATION OF SARCOMA

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**Background:** Soft tissue malignancies, other than rhabdomyosarcoma, are uncommon in infancy, representing 6% of childhood malignancies.

**Objectives:** Describe a challenging case of a newborn with an arm mass.

**Method:** Medical record review.

**Results:** A female infant was referred to hematology for a congenital, rapidly enlarging arm mass. Imaging studies showed a highly extensive, infiltrative mass involving multiple spaces of the left upper extremity, neck, and chest which was highly suggestive of a Kaposiform hemangioendothelioma (KHE) and the patient was started on steroids and vincristine. There was no evidence of Kasabach-Merritt phenomenon (KMP) despite the impressive size. Due to uncertainty of diagnosis without KMP, a biopsy of the left forearm was performed which was reported as hemangioendothelioma, with some features of infantile lipofibromatosis. A second biopsy of a central portion of the lesion was consistent with congenital lipofibromatosis with positive PROX-1 immunostaining; hence treatment was changed to sirolimus. Clinically the mass did not show much improvement and three months into her treatment course she was noted to develop a new mass on her left back/axilla. An excisional biopsy was performed and pathology demonstrated sarcoma surrounded by areas of lipofibromatosis.

**Conclusion:** This case demonstrates the importance of a broad differential of a congenital arm mass. Although initial imaging showed a highly vascular mass, without KMP, the diagnosis of KHE was always doubtful. Profuse vascularity of the mass with high surgical risk of hemorrhage hindered the ability to obtain a good biopsy specimen. The patient was started on chemotherapy for infantile fibrosarcoma, however was found to be translocation negative. Despite this, the patient has shown good response to treatment, and now carries the diagnosis of undifferentiated sarcoma. Genomic profiling showed that the patient has a mutation in chromosome 11p15.5 with loss of heterozygosity and imprinting, which is associated with Beckwith-Wiedemann syndrome (BWS). BWS is a congenital overgrowth syndrome with an increased risk of developing childhood tumors. With this new finding of chromosome 11 mutations, the patient's diagnosis of sarcoma is clearer, however, this case demonstrates challenging radiological and pathologic features. To date, there are no documented case reports of lipofibromatosis transforming into sarcoma.

## SEVERE, PERSISTENT AND FATAL T-CELL IMMUNODEFICIENCY POST INFANTILE LEUKEMIA THERAPY

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**Background:** Intensification of therapy for infants with acute lymphoblastic leukemia (ALL) has resulted in fewer relapses, but a concomitant increase in deaths during induction therapy. Severe, non-HIV acquired immunodeficiency persisting after therapy has not been described as complication.

**Objectives:** To alert clinicians to the risk of severe persistent T-cell immunodeficiency following intensive infant ALL therapy.

**Design/Method:** Case report of two children with persistent thymic involution following treatment for infantile ALL.

**Results:** Both patients were previously healthy 5 month old infants diagnosed with MLL-rearranged ALL and treated with Children's Oncology Group AALL0631 protocol, arm B, based on the P9407 study. Neither received the experimental therapy, lestaurtanib. Both were HIV negative at diagnosis and follow-up. Patient A's treatment was complicated with intermittent infections including *Mycobacterium chelonae* cellulitis. After completing therapy he developed persistent fevers, diarrhea and had multiple infections, including cytomegalovirus, *Clostridium difficile*, and *M. chelonae*. One month after therapy completion, he was found to have a severe CD4 T-cell deficiency, absolute CD4 count 4 cells/ $\mu$ L (normal range 700-2200 cells/ $\mu$ L). One month later he died with disseminated Aspergillosis, severe thymic involution and lymphoid depletion identified on autopsy. Patient B, native Canadian descent, had a family history of consanguinity. During treatment she had multiple infections, enteritis, extensive thrombi involving her upper venous system and prolonged neutropenia. At the end of treatment she developed fevers and diarrhea. She remained in hospital due to ongoing and recurrent infections, including oral herpes simplex virus, *Clostridium difficile*, human herpesvirus 6, bocavirus, rhinovirus and norovirus, despite a normal lymphocyte count. She was diagnosed with severe CD4 T-cell deficiency, CD4 count 14 cells/ $\mu$ L that persisted despite a trial of interleukin 7 therapy. Testing for RAG2 mutations found in this community was negative. She died six months post-therapy, with diffuse bronchopneumonia, severe thymic involution and marked lymphoid depletion on autopsy.

**Conclusion:** These cases represent infants with severe persistent, chemotherapy-induced immunodeficiency. Some infants appear exquisitely sensitive to infantile ALL therapy and clinicians should be alert to the possibility of persistent T-cell deficiency post-therapy.

Poster # 652

## HEPATOBLASTOMA ASSOCIATED WITH CAT EYE SYNDROME AND CONGENITAL UROGENITAL ANOMALIES

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**Background:** Hepatoblastoma has been associated with several risk factors, most notably low birth weight, young maternal age, and small gestational age, early exposure to oxygen administration and furosemide, and genetic abnormalities. Beckwith-Wiedemann Syndrome and Familial-Adenomatous-Polyposis are the two most firmly established genetic syndromes associated with hepatoblastoma, though there are reported cases of hepatoblastoma in children with other genetic syndromes.

**Objectives:** Describe two cases of hepatoblastoma in the background of congenital anomalies.

**Design/Method:** Case series

**Results:** Case 1: 3-year old male with known history of Cat-Eye-Syndrome (partial tetrasomy of chromosome 22q11.1-q11.21), developmental delay, TAPVR, failure to thrive, right ear pit, mid-face hypoplasia, Duane anomaly, microcephaly, and history of full-term birth but low birth weight (2705g) was found to have a liver mass. His AFP was 18,710 ng/ml and a liver biopsy proved hepatoblastoma (non-PFH). PRETEXT was 3, and after 4 cycles of chemotherapy with Vincristine, 5-Fluorouracil and Cisplatin (Doxorubicin was omitted due to complex heart disease), POST-TEXT was 2 and he underwent wide surgical resection. AFP after 4 cycles of chemotherapy and prior to surgery was 4,999 ng/mL. Only one cycle of chemotherapy post-surgery was given due to cardiac complications. Case 2: 20-month old female with history of prematurity (27 weeks gestation), low birth weight (1210 g), congenital cloacal anomaly (ambiguous genitalia, confluence of the anus, bladder, and uterus, s/p colostomy/urostomy), dysmorphism and CGH array showing a 481.89kb loss of chromosome 10q26.2 presented with constipation and was diagnosed with metastatic hepatoblastoma (non-PFH). Diagnostic AFP was 152,000 ng/mL. PRETEXT was 4 and after 4 cycles of chemotherapy with Vincristine, 5-Fluorouracil, Cisplatin and Doxorubicin, POST-TEXT was 2. She underwent wide surgical resection. AFP declined to 3,588 ng/mL after two cycles of chemotherapy and 682 ng/mL prior to surgery. Patient is doing well with no evidence of disease 20 months after completion of therapy.

**Conclusion:** The association of hepatoblastoma and kidney/bladder abnormalities has recently been reported, although the association of loss of chromosome 10 has not been reported before. To our knowledge this is also the first report of hepatoblastoma associated with Cat Eye Syndrome.

Poster # 653

## **HISTIOCYTIC SARCOMA, JUVENILE XANTHOGRANULOMA, NEUROFIBROMAS AND B-ALL IN A PATIENT WITH CONSTITUTIONAL HOMOZYGOUS DELETION WITHIN CDKN2A**

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**Background:** Histiocytic sarcoma (HS) is a rare malignancy, particularly uncommon in children. The etiology of HS is unknown, but it has been associated with loss of the tumor suppressor, p16<sup>INK4A</sup>, in a murine model and in human tumors. Deletion of the CDKN2A locus encoding p16<sup>INK4A</sup> is seen in acute lymphoblastic leukemia (ALL) and mutations in CDKN2A have been identified in family kindreds with melanoma, breast cancer, pancreatic cancer and neurofibromas known as familial atypical malignant melanoma (FAMMM) syndrome.

**Objectives:** Report a patient with constitutional homozygous 14 bp deletion of exon 2 of CDKN2A and juvenile xanthogranuloma (JXG), HS, neurofibromas and B-ALL.

**Design/Method:** Case Report.

**Results:** 12 year-old female with a past medical history of JXG, presented with a rapidly growing mass on her left hand. Excisional biopsy revealed HS, positive for CD68 and CD45 while negative for B lineage markers. She completed treatment per AIEOP ALCL99 protocol with no recurrence to date. Two and a half years after completion of therapy, the patient now 16, presented with anemia, thrombocytopenia and leukocytosis with 80% blasts on the peripheral smear. Bone marrow evaluation revealed 89% blasts with progenitor B cell phenotype consistent with precursor B-ALL. Cytogenetic studies revealed t(8;14)(q11.2;q32), a non-myc translocation



observed in B-ALL with FISH negative for t(9;22), t(8;21) and 11q23 (MLL) translocation. The patient was diagnosed with very high risk (VHR) Precursor B-ALL and she started on therapy as per Children's Oncology Group protocol AALL 1131. She is in remission and still on therapy 18 months later. Concurrently, the patient was found to have multiple neurofibromas (biopsy proven) and spongiotic changes on her brain MRI. A cancer predisposition syndrome work up was undertaken. Genetic testing for NF-1 and PTEN were negative. However, DNA sequencing from blood revealed a homozygous 14 bp deletion in exon 2 of CDKN2A. Buccal testing also revealed the same homozygous deletion within CDKN2A consistent with FAMMM syndrome. **Conclusion:** We present a patient with HS, B-ALL, JXG and neurofibromas with constitutional homozygous deletion within CDKN2A. The findings indicate that the cancer predisposition associated with germline CDKN2A mutations in FAMMM syndrome may include neoplasms of histiocytic, lymphoid and nervous system origin as well as melanoma.

Poster # 654

## **TWO RARE CASES OF HEPATOCELLULAR MALIGNANT NEOPLASM (HMN) - NOT OTHERWISE SPECIFIED (NOS), A NEWLY RECOGNIZED PEDIATRIC CANCER**

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**Background:** The most common malignant pediatric hepatocellular tumors are hepatoblastoma (HB) and hepatocellular carcinoma (HCC). Complicated histologic variances make a definitive diagnosis often difficult yet extremely important in determining appropriate therapy. A small subset of malignant hepatocellular tumors has recently gained recognition in older children/young adolescents, possessing mixed histological patterns typical of both HB and HCC. Recognizing the existence of these tumors will help guide therapy and impact eligibility for liver transplant.

**Objectives:** Describe two rare cases of Hepatocellular Malignant Neoplasm-NOS

**Design/Method:** Case Series

**Results: Patient #1:** 14 y.o. male without previous liver disease presented with large heterogeneous multi-lobulated hepatic mass, local metastatic lesions (pretext-IV), AFP level 38,583ng/mL. Liver biopsy pathology report yielded diagnosis of HB (strongly and diffusely positive for HEPR and Glypican-3, beta-catenin membranous staining, CK8/18 negative). Based on rarity of tumor for age, second opinion was obtained, pathology results revealing contradicting diagnosis of HCC. With rising AFP 281,285ng/mL and worsening LFTs (AST275, ALT470), cisplatin/fluorouracil/vincristine/doxorubicin therapy according to COG protocol AHEP 0731, Regimen F was started. While showing rapid clinical improvement, third opinion was obtained, pathology determined to be HMN-NOS. Fourth opinion concurred. Based on continued clinical response yet remaining unresectable tumor, liver transplant is planned following 4 cycles of cisplatin/fluorouracil/vincristine/doxorubicin therapy. **Patient#2:** 8 y.o female without previous liver disease presented with extensive multifocal heterogenous hepatic disease (pretext-IV), AFP level 800,000ng/mL, associated 3mm right lung base noncalcified nodule. Liver core biopsy yielded diagnosis of HB (membranous and cytoplasmic staining positive for beta-catenin with only patchy nuclear positivity for p53, variable S-100). Based on contradicting impressions, second opinion was obtained, immunochemistry predominantly favoring HB, though some areas favoring HCC. Third opinion concluded HCC. Awaiting

transplant evaluation, AFP rose to  $>1.1 \times 10^6$  ng/ml. Transplanting institution determined diagnosis of HMN-NOS and started treatment with cisplatin/doxorubin/sorafenib, associated malignant fevers and pain resolved after her first cycle. Sorafenib not well tolerated, treatment continued with just cisplatin/doxorubicin. After 4 cycles, AFP level (33,000 ng/mL) and liver size were significantly reduced. Patient remains a liver transplant candidate, currently awaiting surgery.

**Conclusion:** Hepatocellular Malignant Neoplasm-NOS is a rare new classification of transplantable pediatric hepatocellular tumor requiring further definitive histologic characterization and therapy response assessment.

Poster # 655

## **LARGE GRANULAR LYMPHOCYTE LEUKEMIA PRESENTING IN ADOLESCENCE**

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**Background:** Large granular lymphocyte (LGL) leukemia is a mature T-cell neoplasm characterized by inappropriate expansion of LGL cells of T-cell or natural killer cell origin. While considered indolent, most patients demonstrate cytopenias, recurrent infections, splenomegaly and autoimmunity. LGL leukemia affects older adults with a median age of 60 years. Pediatric LGL leukemia is rarely reported.

**Objective:** We describe a case of T-cell LGL leukemia in an adolescent female who presented with fatigue, weight loss, lymphadenopathy, severe neutropenia and recurrent abscesses.

**Methods:** Peripheral blood and bone marrow features of T-cell LGL leukemia were demonstrated via flow cytometry and specific immunohistochemistry staining. Clonality was documented by T-cell receptor (TCR)  $\beta/\gamma$  gene rearrangement analysis using polymerase chain reaction (PCR).

**Results:** A 17-year-old previously healthy female was referred for evaluation of fatigue, weight loss, submandibular/cervical lymphadenopathy, severe neutropenia and recurrent labial abscesses. Hepatomegaly without splenomegaly was noted. Evaluation of peripheral blood demonstrated reactive lymphocytosis. Excisional biopsies of a labial abscess and a cervical lymph node demonstrated lymphoid aggregates and lymphoid hyperplasia. Bone marrow examination demonstrated a hypocellular marrow (60%) with trilineage hematopoiesis, and immunohistochemistry highlighted T-cell infiltrates positive for CD8, granzyme B, and TIA-1. Cytogenetic evaluation documented a normal female karyotype without numerical/structural aberrations. TCR  $\beta/\gamma$  gene rearrangement studies by PCR were positive for a clonal population of T cells consistent with T-cell LGL leukemia. Moreover, flow cytometry demonstrated the presence of CD3<sup>+</sup>/CD8<sup>+</sup>/CD57<sup>+</sup> lymphocytes (41%). Infectious etiologies and other immune dysregulation states were excluded. Her neutropenia and labial abscesses were unresponsive to antibiotics and granulocyte stimulating agents prompting the initiation of Immunosuppressive therapy with oral methotrexate (10mg/m<sup>2</sup>/week). Four months later, she remained unresponsive to therapy as demonstrated by ongoing severe neutropenia, adenopathy, labial abscesses, and persistence of the T-cell clone. Immunosuppressive therapy was changed to oral cyclophosphamide (100mg/day).

**Conclusion:** The rarity of LGL leukemia in an adolescent makes timely diagnosis a challenge. Nonetheless, a diagnosis of LGL leukemia should be considered in a patient with a consistent clinical phenotype including cytopenias, recurrent infections, splenomegaly and autoimmunity.

Achieving successful disease control remains challenging due to the lack of consensus in the treatment of LGL disorders.

Poster # 656

### **CASE REPORT: THE DIAGNOSIS AND TREATMENT OF PRIMARY MYELOFIBROSIS IN TWIN ADOLESCENTS**

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**Background:** Pediatric primary myelofibrosis is a rare myeloproliferative neoplasm that results in unregulated over expression of marrow collagen. Without standardized diagnostic or treatment guidelines, the mortality for this disorder remains high. Unlike adult myelofibrosis, a common genetic basis for pediatric primary myelofibrosis has yet to be identified.

**Objectives:** We report a case of twin adolescent males found to have extensive marrow fibrosis on magnetic resonance imaging (MRI). Their evaluation questions the genetic basis of pediatric primary myelofibrosis and their management further debates the role of corticosteroids as a less aggressive treatment strategy.

**Design/Method:** Review of the subjects' medical charts and literature search using PubMed.

**Results:** Twin A presented at 16 years of age with splenomegaly and pancytopenia. Abdominal MRI revealed diffuse low T1 and T2 signal intensity in vertebral and pelvic bone marrow. Bone marrow analysis demonstrated hypercellularity with lymphoid aggregates and markedly positive reticulin stain. Evaluation for JAK2-V617F, MPL and CALR mutations was negative. Twin B was evaluated as a possible marrow donor. Although clinically well, evaluation revealed mild thrombocytopenia, marrow hypocellularity and a moderately positive reticulin stain. Abdominal MRI showed diffuse low signal intensity in the identical distribution to his twin. Twin A was treated with daily prednisone (2mg/kg/day) with gradual clinical and symptomatic resolution of cytopenias and splenomegaly.

**Conclusion:** Pediatric and adult myelofibrosis are separate conditions whose genetic predisposition and clinical outcomes are unique. The hematologic, marrow and imaging abnormalities shared by our twin siblings make it difficult to ignore the likely genetic predisposition they share. Further genomic sequencing studies will be required. The use of MRI to diagnosis and monitor pediatric myelofibrosis provides superior bone marrow analysis and may overcome sampling errors experienced with bone marrow biopsy alone. Supportive care may be appropriate in mild disease. Corticosteroids may induce a clinical remission in more symptomatic disease; however, they do not appear to reverse marrow fibrosis.

Poster # 657

### **CASE REPORT: SUCCESSFUL CONCURRENT TREATMENT OF CHRONIC HEPATITIS B INFECTION IN PEDIATRIC B CELL ACUTE LYMPHOBLASTIC LEUKEMIA AND A ROLE FOR SCREENING OF HIGH RISK CARRIERS**

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**Background:** HBV reactivation can occur in patients during periods of intense immune suppression. Chronic HBV infection is often asymptomatic and may be under-recognized at initiation of multi-agent chemotherapy even in high risk populations. Guidelines exist for screening of high risk carriers for hepatitis B. However, aside from screening for hepatitis B prior to the use of monoclonal antibodies such as Rituximab this does not constitute routine practice for other pediatric malignancies and can be overlooked in the initial evaluation of a critically sick child about to begin induction chemotherapy.

**Objectives:** The goal of this report is to highlight that hepatitis B screening should be considered in high risk children at the start of induction chemotherapy and that a high hepatitis B viral load does not preclude successful treatment of the malignancy if appropriate anti-viral treatment is given concurrently

**Design/Method:** Retrospective case report of a single patient's chart with no patient identifiers.

**Results:** We report the serendipitous discovery of asymptomatic hepatitis B infection in a 6 year old male with precursor B acute lymphoblastic leukemia. The child presented with abdominal pain during induction chemotherapy that was due to drug induced pancreatitis. Elevated liver enzymes prompted testing for hepatitis serology. He was a recent immigrant from an area of high prevalence and his hepatitis B was vertically transmitted. Our patient had a very high viral load at diagnosis. He began treatment with Lamivudine but was switched to Entecavir with a rapid decline in viral load. Chemotherapy was administered in a timely fashion with no dose modifications. He is currently free of leukemia.

**Conclusion:** We illustrate that concurrent successful treatment of both acute lymphoblastic leukemia and asymptomatic hepatitis B is feasible in the pediatric setting and may not require dose modification of chemotherapy. This report also highlights that testing for asymptomatic Hepatitis B carrier status in patients that belong to high risk populations ( eg. children born in areas or to parents that have immigrated from areas with > 2 % prevalence of hepatitis B ) will help to identify asymptomatic chronic carriers and start timely anti-viral treatment.

Poster # 658

## SECONDARY VIP-SECRETION IN INTERMEDIATE RISK NEUROBLASTOMA

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**Background:** Vasoactive intestinal peptide (VIP) secretion has been described as a rare paraneoplastic syndrome, occurring in less than 1% of patients with neuroblastoma. Most reported cases are of primary VIP-secreting neuroblastoma wherein the syndrome is present at the time of diagnosis. However, there have been six reported cases of neuroblastoma which exhibit secondary VIP-secretion - developing after initiation of chemotherapy. All of these cases were high-risk at the time of diagnosis.

**Objectives:** Here we present a novel case of an intermediate-risk neuroblastoma exhibiting secondary VIP-secretion.

**Design/Method:** All patient care was carried out at Children's Hospital Colorado and data was collected by a retrospective chart review.

**Results:** An 11 month-old female presented with a history of abdominal pain and constipation. Imaging revealed a large pelvic mass with extension into the spinal canal and through the left sciatic notch. The tumor was MIBG-avid and further work-up revealed no distant metastases or bone marrow involvement. Biopsy revealed poorly-differentiated neuroblastoma with unfavorable histology, MYCN non-amplified. Chemotherapy was initiated for treatment of stage

III neuroblastoma. Two months after diagnosis the patient developed diarrhea, a 10% weight loss, and hypokalemia (potassium of 1.7mmol/L). Laboratory evaluation revealed a VIP level greater than 20-times the upper limit of normal. She was started on an octreotide infusion in an attempt to allay symptoms, but continued to have profuse diarrhea and refractory hypokalemia so was taken to the OR for tumor debulking. The surgical team achieved a >90% tumor resection resulting in resolution of diarrhea and normalization of serum potassium and VIP by POD#4. The patient suffered a localized relapse 6 months off therapy and died of disease 18 months from initial diagnosis. She had no recurrence of her paraneoplastic syndrome.

**Conclusion:** This case describes a patient with an intermediate-risk neuroblastoma with secondary VIP-secretion. This patient was unsuccessfully treated with a somatostatin analog, consistent with two previously reported cases. Secondary VIP-secretion has previously been reported only in high-risk tumors; therefore when present in lower-risk disease as presented here may portend a poor outcome. In addition, this case highlights the importance of surgical intervention as the only known means of symptomatic control.

Poster # 659

## **PTOSIS CAUSED BY LACRIMAL GLAND INFILTRATION AS A PRESENTING SIGN IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)**

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**Background:** ALL is the most common pediatric malignancy and may involve the Central Nervous System (CNS). CNS leukemia increases risk of relapse and requires additional, potentially toxic therapy. Accurate diagnosis of CNS leukemia is essential to risk stratification.

**Objectives:** Describe a case of ALL with ptosis caused by lacrimal gland infiltration, review the literature, and discuss implications for risk stratification.

**Design/Method:** Case Report

**Results:** A previously healthy 4-year-old male presented with several days of fever and limp as well as several weeks of right eye ptosis. He was found to be pancytopenic and a diagnosis of ALL was made by peripheral blood flow cytometry. Given the finding of asymmetric ptosis on exam, CNS leukemia was suspected and a neurology consult and MRI of the head were obtained in addition to the standard lumbar puncture (LP). Neurology noted that since pupillary function and eye movements were unaffected, the ptosis was unlikely to be caused by third cranial nerve involvement. Indeed, MRI of the brain was normal; no abnormal enhancement was seen along the third cranial nerve. The MRI did, however, reveal enlargement of the right lacrimal gland with associated intraorbital mass effect thought to be due to leukemic infiltrate. The LP showed no evidence of CNS leukemia. The patient was classified as CNS 1 and began induction. The ptosis resolved on day 3 of induction. A comprehensive literature review revealed that leukemic infiltrate of the lacrimal gland is an uncommon finding in ALL, with three prior case reports: One at diagnosis with bilateral involvement and concomitant retinopathy; and two at relapse.

**Conclusion:** Lacrimal gland infiltration can be a cause of ptosis at diagnosis of ALL, resulting in exam findings that may mimic CNS leukemia. Careful examination and imaging is recommended to properly risk stratify patients.

Poster # 660

# **CEREBRAL METASTASIS OF HEPATOBLASTOMA: WHAT IS THE BEST THERAPY?**

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**Background:** Hepatoblastoma (HB) is the most common primary liver tumor of childhood. Cerebral metastasis is a rarely reported site of disease and the best mode of treatment is not defined. Resection appears beneficial for solitary lesions, but may not be feasible for multifocal disease. The role of radiation and other therapy in such patients is unclear.

**Objectives:** Describe a case of HB with brain metastasis and review the medical literature for similar cases in an attempt to develop rational treatment recommendations.

**Design/Method:** Case report via chart review, literature search via MEDLINE (search terms of “hepatoblastoma”, “relapse”, “cerebral”, “treatment”) and manual review of bibliographies of published reports of HB.

**Results:** Our patient is an 8-year-old male, diagnosed with HB metastatic to both lungs in 2009. Initial treatment consisted of 6 cycles of “CV5D” chemotherapy sandwiched around resection of his primary and metastatic disease. Over the next 4 years he sustained multiple pulmonary relapses, treated with thoroscopic resections plus 4 separate chemotherapy regimens (combinations of irinotecan, vincristine, carboplatin, etoposide, oxaloplatin, gemcitabine, and cisplatin). His disease showed a minor but sustained response to bevacizumab and sorafenib before progressing in the lungs and liver in January 2014. He achieved a partial remission to pazopanib. Following a pulmonary lobectomy he developed depressed mental status, aphasia, and right-sided hemiplegia. Neuroimaging revealed multiple hemorrhagic foci centered on metastatic nodules in the left frontal and right parietal lobes. He was treated with cerebral radiation therapy (3000 cGy in 10 fractions) and temozolomide, demonstrating stable disease, a transient drop in AFP, and improvement of his neurological symptoms. At 3 months post brain metastasis he remains out of hospital with a Lansky score of 60.

Our preliminary literature search identified 22 articles describing 37 patients with HB metastatic to the brain. These patient’s demographics, treatments, and outcomes will be presented.

**Conclusion:** We report a child with HB metastatic to the brain, treated with radiation and temozolomide. Further research and experience are necessary to define the best management of such children. We will present the results of our extensive literature review as a first step in this effort.

Poster # 661

# **ACUTE ERYTHROID LEUKEMIA AND PULMONARY FIBROSIS: A CASE REPORT AND REVIEW OF THE LITERATURE**

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**Background:** Acute Erythroid Leukemia (AEL) is a rare subtype of Acute Myeloid Leukemia in which erythroid cells comprise at least 50% of all cells and myeloblasts represent at least 20% of the nonerythroid cells. Diagnosis may be difficult due to its rarity as well as morphologic

similarities with myelodysplastic syndrome and reactive erythroid hyperplasia. Pediatric AEL is exceedingly uncommon in the literature, and prognosis appears to be very poor.

**Objectives:** We report on a pediatric patient diagnosed with AEL who subsequently developed respiratory failure with pulmonary fibrosis.

**Design/Method:** A 7-year-old female with a history of short stature and failure to thrive presented with pancytopenia and fevers. Bone marrow evaluation was consistent with AEL with erythroid predominance (85% of cells) with various stages of maturation and a small population of myeloblasts. She underwent an evaluation for genetic predisposition syndromes. DEB testing was negative for Fanconi Anemia. Telomere lengths were less than tenth percentile, but genetic testing for Dykeratosis Congenita was negative. A PTPN11 T570k mosaic mutation was identified for Noonan Syndrome.

**Results:** Prior to initiation of chemotherapy, she developed respiratory failure with diffuse alveolar and interstitial opacities on chest CT requiring intubation. Due to persistent respiratory failure and significant abnormalities on CT, bronchoalveolar lavage and lung biopsy were performed that showed extensive interstitial and septal fibrosis but no evidence of infection. A specific etiology for her pulmonary disease was never identified but hypothesized to be secondary to leukemic infiltrate and subsequent inflammatory response as it improved throughout leukemia therapy. She attained remission after her first cycle of ADE chemotherapy. Although she had a matched related donor for bone marrow transplantation, the potential transplant-associated morbidity and mortality were considered too great secondary to her pulmonary disease. She was treated with chemotherapy only and remains in remission eight months after diagnosis.

**Conclusion:** AEL is rare, particularly in children, which makes determining risk factors and prognosis as well as the best treatment modality difficult, but it appears to have a worse prognosis than AML in general. This is the first report of its association with pulmonary fibrosis, although the etiology or association is unclear.

Poster # 662

## **A RARE CASE OF A TESTICULAR LEYDIG CELL TUMOR IN A 4-YEAR-OLD BOY WHO PRESENTED WITH ISOSEXUAL PRECOCIOUS PUBERTY AND A UNILATERAL TESTICULAR MASS**

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**Background:** Leydig cell tumors are rare stromal testicular tumors found most often in adults. We describe an unusual case of a Leydig cell tumor in a 4-year-old patient and review the literature about pre-pubertal diagnoses with a focus on optimal management to preserve fertility.

**Objectives:** To increase index of suspicion in children with precocious puberty or virilization. Leydig cell tumors are benign in children, however early detection and treatment are important to prevent central precocious puberty and impaired growth and to preserve reproductive function.

**Design/Method:** A 4-year-old male presented with a right testicular mass and isosexual precocious puberty. The parents first reported growth acceleration followed by penile enlargement, appearance of pubic hair, facial acne, and adult body odor. He presented five months after symptom onset and examination revealed height above the 99<sup>th</sup> percentile, a deep voice, Tanner III pubic hair, and a firm non-tender right testicular mass. Serum testosterone was

highly elevated (462 ng/dL) along with calcium and alkaline phosphatase likely secondary to testosterone effect on the bone. Lactate dehydrogenase, and conventional tumor markers serum AFP and serum Beta-hCG were normal. Testicular ultrasonography confirmed a well-circumscribed relatively hypoechoic 2-cm mass. His bone age was increased to a mean of 12 years.

**Results:** The patient underwent a partial orchiectomy with complete tumor excision. Pathology was consistent with a Leydig cell tumor without malignant features. One week later his symptoms were improving and testosterone decreased to  $< 3$  ng/dL. We report on the limited pre-pubertal cases in the literature; previously conventional treatment involved radical orchiectomy. Our patient is tumor-free three months after surgery with no evidence of recurrence or central precocious puberty.

**Conclusion:** Leydig cell tumors can be seen in pre-pubertal children. We describe one of the youngest reported cases of this benign diagnosis in children. Excess steroid hormones, particularly androgens, result in symptoms of sexual precocity and virilization. Even if no testicular mass is palpable, ultrasonography can help to detect these tumors that can then be confirmed with laboratory evaluation. Complete tumor excision via partial orchiectomy should be considered when surgically feasible as it will achieve resolution of reversible clinical findings and preserve optimal fertility.

Poster # 663

## **A COMMON PRESENTATION OF A VERY RARE PEDIATRIC ENTITY: T-CELL LARGE GRANULAR LYMPHOCYTIC LEUKEMIA IN A CHILD**

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**Background:** Large granular lymphocyte (LGL) leukemia is a very rare clonal proliferation of either CD3- cytotoxic T or CD3-NK cells which results in anemia, neutropenia and /or thrombocytopenia. Median age at diagnosis is 60 years and the disease is associated in almost always with autoimmune and lymphoproliferative disorders. There are  $< 5$  reported cases of T-LGL in pediatrics age group and none to our knowledge in a previously healthy child.

**Objectives:** To report a rare case of T –LGL leukemia in a 6 year old child

**Case report:** 6 year old male was evaluated for persistent worsening macrocytic anemia with mild leukopenia of 18 month duration. Extensive negative work up for various causes of anemia, followed by bone marrow aspirate and biopsy revealed a infiltration by clonal ( positive TCR rearrangement ) atypical T-lymphocytic population (CD3+/CD8+/CD57+ by flow cytometry), consistent with LGL Leukemia. Follow up over 6 months showed persistent severe anemia with restriction in physical activity and deterioration in school performance. Repeat bone-marrow aspirate revealed increase in the clonal population with features of T- LGL. Work up for rheumatoid diseases and lymphoproliferative diseases were negative. PET was negative for focal lymphoid proliferation. Treatment with 10 mg/m<sup>2</sup> oral methotrexate once a week was initiated.

**Results:** In this unusual clonal T cell proliferation in a 6 year old child, the differential includes an abnormal immunological disorder/response versus T-LGL leukemia. The latter is very unusual in such a young patient. The immunophenotypic and morphologic (marrow sinusoidal infiltration, sinus dilatation) findings combined with the clinical features are consistent with T-LGL leukemia. The disease is indolent with majority requiring treatment within 10 years of diagnosis. Dysregulated apoptosis has been shown to be an important mechanism in the



pathogenesis of LGL leukemia. No standard therapy has been established. Immunosuppressive therapy remains the foundation of treatment which can control but not cure the disease.

**Conclusion:** This is an extremely rare but important diagnosis to consider in the work up of a pediatric patient with persistent anemia and leukopenia with expansion of LGL's and careful pathologic analysis and clinicopathologic correlation is essential for diagnosis.

Poster # 664

## **ADRENAL INSUFFICIENCY IN PATIENTS WITH HIGH RISK NEUROBLASTOMA**

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**Background:** Neuroblastoma represents the most common extracranial solid tumor of childhood and can be associated with many treatment complications and late effects in those with high risk disease. The chemotherapeutic agents used to treat neuroblastoma are associated with musculoskeletal, neurologic, endocrinologic and sensory complications. Adrenal insufficiency has not been reported. In pediatric oncology patients, transient adrenal insufficiency is reported as a result of hypotensive critical illness. We reviewed two patients with high risk neuroblastoma who developed adrenal insufficiency while on therapy.

**Objectives:** To identify the clinical presentation, diagnosis and treatment of adrenal insufficiency in patients with high risk neuroblastoma.

**Design/Method:** A single institution chart review was performed looking at patients diagnosed with high risk neuroblastoma who developed adrenal insufficiency in 2014. A detailed review of the clinical presentation, measurement of cortisol levels, and course of therapy for those affected were summarized as a case report.

**Results:** Of the 6 patients diagnosed with high-risk neuroblastoma 2 developed adrenal insufficiency. Characteristics common to both patients were: both were female, less than 2 years old at diagnosis and received same therapy including left adrenalectomy. Both presented in hypotensive crisis when adrenal insufficiency was diagnosed. The first patient presented with fluid refractory hypotensive septic shock, and blood cultures grew *Burkholderia cepacia* complex. The second patient presented with fluid refractory shock during cytokine and immunotherapy. In both patients, adrenal insufficiency was diagnosed based on a morning cortisol level of <1.0mcg/dL. Both patients underwent a full endocrine work-up and were placed on physiologic hydrocortisone therapy. They have since remained hemodynamically stable despite ongoing neuroblastoma therapy. No identified predisposing factors were present.

**Conclusion:** A review of the current literature found no cases of adrenal insufficiency associated with neuroblastoma or its treatment. Hypotension was the primary factor that lead to evaluation for and treatment of adrenal insufficiency in these two cases. The intensity of therapy required for high risk neuroblastoma and these two cases raise the question of whether a baseline evaluation of adrenal function should be part of the standard pre-therapy or post-induction organ assessment.

Poster # 665

## **CO-EXPRESSION OF MONOSOMY 7 AND CHROMOSOME 3 ABNORMALITY IN PEDIATRIC ACUTE MYELOID LEUKEMIA (AML) PRESENTING WITH CENTRAL DIABETES INSIPIDUS (DI) IS RARE AND HAS A POOR PROGNOSIS**

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**Background:** DI is a rare presentation of AML and is frequently associated with monosomy 7. Concomitant chromosome 3 abnormalities are reported in adults with monosomy 7 positive AML and DI and confer a poor prognosis.

**Objectives:** Pediatric literature on monosomy 7 AML with DI is scanty. The significance of concomitant chromosome 3 abnormalities remains undetermined.

**Design/Method:** Describe an unusual case of childhood AML presenting very early with DI, characterized by monosomy 7 and t(3;3) ( previously unreported ) and review previously reported pediatric AML with monosomy 7 and DI.

**Results:** A 7-year-old girl presenting with polydipsia, polyuria, hypernatremia, dilute urine and serum hyperosmolality was diagnosed with idiopathic DI. Six months later, she developed bony pains, fever, lymphadenopathy, hepatosplenomegaly, leukocytosis (15.9 K/uL), anemia (5.8g/dL), thrombocytopenia (57 K/uL) and circulating blasts. Bone marrow was consistent with AML and chromosomes confirmed monosomy 7 and t(3;3)(q21;q26.2). Remission was not achieved despite two induction cycles and salvage chemotherapy. Decitabine was administered, followed by a matched unrelated umbilical cord-blood transplant. Preparative therapy comprised total body irradiation, fludarabine, melphalan, and rATG. She engrafted with 98% donor chimerism but died three months later from adenoviremia, pneumonia, and reappearance of AML blasts.

**Conclusion:** Similar to adults, pediatric AML with DI is strongly associated with monosomy 7 and may harbor concomitant chromosome 3 anomalies. We review five cases with documented cytogenetics, from eleven reported pediatric cases of DI in myeloid malignancies and report one presenting with monosomy 7 and t(3;3), illustrating that this subpopulation may warrant alternative and aggressive therapeutic options.

Table: Characteristics of previously reported pediatric monosomy-7 related myeloid malignancies presenting with Diabetes Insipidus.									
Author (year)	Age (yr)/Sex	Malignancy	Onset of DI from diagnosis	CNS Imaging	Resolution of DI (Days)	Cytogenetics and Immunophenotype	Treatment	Outcome	
Wosman (2002)	11:M	AML (FAB-M7)	At diagnosis	MRI- Normal	80	45XY, inv(3)(q21q26), -7XY; CD41+ and CD61+	MUD-HSCT	Severe GVHD; Dead at 6 months from refractory PCP pneumonia	
Frangou (2000)	11:M	AML (FAB-M2)		MRI-large pituitary; absent T-1 hyperintense signal in posterior pituitary; thick pituitary stalk.	Not resolved	47 XXY; HLA-DR, CD13, and CD33	Chemotherapy Cranial irradiation, CNS therapy	Alive and in remission at 28 months	
Kanubar (1994)	7:M	AML (FAB-M2)	At diagnosis	NA	6	45, XY, 7-, CD2-, CD13-, CD19-, and CD34-	Chemotherapy	Remission not achieved	
Kellen (2003)	6:M	MDS-RAEB	+3 days	MRI- Normal	14	Monosomy 7	MUD-HSCT	Alive in remission	
Sungpolchai (2013)	8:F	JMML		MRI- absent bright spot over posterior pituitary.	NA	Monosomy 7	On DDAVP	Awaiting donor for HSCT	
Present Case	7:F	AML	-6 months	MRI- Normal	41	Monosomy 7 (-7) t(3;3)(q21;q26.2) CD45+, CD13+, CD33+, CD117+, CD11c+, HLA-DR+	Umbilical cord -HSCT	Relapsed day 79 of allo-HSCT. Died from disseminated adenoviremia	

Poster # 666

## NOVEL ASSOCIATION OF FAMILIAL TESTICULAR GERM CELL TUMOR AND AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE WITH PKD1 MUTATION

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**Background:** Familial testicular germ cell tumors (FTGCT) have been well described and thought to be secondary to the combined effect of multiple, common, low-penetrance risk alleles. Several SNPs along with one microdeletion have been implicated as risk alleles including *PKD1*. Autosomal dominant polycystic kidney disease (ADPKD) is known to be associated with *PKD1* and *PKD2* mutations. *PKD1* encodes the protein polycystin-1 thought to play a role in cell growth, proliferation, migration, and cell-to-cell interactions in ciliated epithelia including the testes. We describe two brothers diagnosed in the same month with FTGCT and both subsequently found to have polycystic kidney disease on imaging obtained for disease staging.

**Objectives:** To describe the association of FTGCT and ADPKD in this family.

**Design/Method:** Family history with a four-generation pedigree was obtained. Clinical exome sequencing (CES) at the UCLA Clinical Genomics Center was performed on peripheral blood from the two brothers as well as their mother. The father was not available for testing and his medical history is unknown.

**Results:** CES identified, in both brothers, a maternally inherited c.1723-1G>A variant predicted to disrupt the canonical splice acceptor site prior to exon 9 of the *PKD1* gene. This variant is novel, as it has not been previously observed in any context including the ExAC database of over 63,000 human exomes.

**Conclusion:** This is the second family ever reported with both FTGCT and ADPKD and the first described association of FTGCT with a splice variant in *PKD1*, located on chromosome 16p13.3. We suggest this variant *PKD1* may convey increased risk for FTGCT in addition to causing ADPKD.

Poster # 667

## **EXTENSIVE PERINEAL AND PERIANAL CONDYLOMA ACUMINATA IN A CHILD UNDERGOING THERAPY FOR ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)**

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**Background:** Condyloma Acuminata (CA) is caused by human papilloma virus (HPV). Giant CA is more commonly reported in the anogenital regions. Its treatment is challenging in the immune compromised patient. No cases of CA treatment in children with leukemia have been previously described.

**Objectives:** We describe our experience managing a patient with ALL and refractory CA.

**Design/Method:** Case Report

**Results:** Six year old female diagnosed with pre-B ALL. At time of diagnosis she had no obvious perianal or perineal lesions. She had no major infectious complications during induction, consolidation or reinduction. During maintenance therapy she developed a perianal condyloma. Topical Imiquimod initially helped control the lesions. Over the next several months the lesions continued to progress. Ultimately she developed confluent verrucous lesions extending from perianal region to anterior of the urethral opening obscuring anal and vaginal orifices. She underwent 3 treatments with Pulsed Dye Laser (585 nm) during maintenance therapy. Oral Cimetidine was initiated. During periods of unexpected neutropenia, the lesions became larger. The absolute lymphocyte count reached greater than 1500 one month after completion of

chemotherapy. As the immune system reconstituted she began to have better response to topical Imiquimod. At 5 months off therapy, the Gardasil vaccine series was initiated. Prior to the fifth laser therapy the lesion had regressed to scattered discrete lesions. Nearly one year after completion of ALL therapy, she received CO2 laser therapy for vaginal condyloma. In situ hybridization of multiple lesions demonstrated presence of low risk HPV and absence of high risk HPV. There has been an excellent response once her counts and immune system recovered. The Gardasil vaccine may have augmented her recovering immune function. Currently, there are only small residual perianal lesions.

**Conclusion:** This case demonstrates extensive CA in a child receiving therapy for ALL. Despite multimodality therapy we were unable to control the CA due to the immunosuppressive effects of ALL therapy. Immune recovery was crucial for resolution of the lesions. Further research is required to determine the role of Gardasil vaccination in improving immune response to CA in children who have received chemotherapy.

Poster # 668

### **ATYPICAL PRESENTATION OF CHEMOTHERAPY INDUCED ACRAL ERYTHEMA (CIAE) AFTER TREATMENT WITH HIGH DOSE METHOTREXATE (HD MTX)**

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**Background:** Chemotherapy induced acral erythema (CIAE) usually presents as bilateral painful erythematous plaques that develop within 3 weeks following chemotherapy.

**Objectives:** We report an atypical presentation of CIAE in a 15 yo female with mixed lineage leukemia and left hemiparesis who developed CIAE in a unilateral distribution over her left elbow, chest wall, third metacarpus, and first metatarsus after receiving high dose (HD) methotrexate (MTX). This is the first report of unilateral presentation of CIAE in pediatric literature.

**Design/Method:** Case report.

**Results:** A 15yo female being treated for mixed lineage leukemia developed a sagittal sinus venous thrombosis and right intraparenchymal hemorrhage resulting in left hemiparesis during Induction. On day 1 of Interim Maintenance she received 5 grams/m<sup>2</sup> of MTX over 24 hours followed by leucovorin (LCV) rescue. Serial MTX levels showed appropriate clearance and her creatinine remained at baseline. 48 hour MTX level was 0.18 µmol/L and per protocol LCV was discontinued at 54 hours. Seventy-two hours after the start of HD MTX she developed pain and tingling in her left elbow and dorsum of her left foot followed by appearance of tender erythematous unilateral plaques over her left elbow, chest wall, third metacarpal, and first metatarsal joint. She also developed severe myelosuppression and mucositis involving her oral mucosa and perianal area. Skin biopsy of her elbow lesion was consistent with CIAE. The lesions completely resolved in 3 weeks. With subsequent HD MTX doses, post-hydration fluid was increased and LCV was continued until MTX level was <0.1 µmol/L. There was no recurrence of CIAE with subsequent doses of HD MTX.

**Conclusion:** Clinicians should be aware of the various dermatologic manifestations in patients undergoing chemotherapy. Differential diagnoses include allergic reaction, contact dermatitis, trauma, cellulitis, abscess, acute GVHD and vasculitis. While the etiology of CIAE is unknown, a direct toxic effect from high concentration of chemotherapy has been implicated as a causative factor. CIAE is typically bilateral in distribution. Our patient preferred a left lateral decubitus

position. We postulate that this may have predisposed her left paretic side to differential blood flow and edema thereby leading to higher MTX concentration and an atypical unilateral distribution of CIAE.

Poster # 669

## **IDENTIFICATION, MANAGEMENT AND OUTCOMES OF ACUTE LEUKEMIAS OF AMBIGUOUS LINEAGE - A CASE SERIES**

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**Background:** Acute leukemias of ambiguous lineage is the term now used to describe what has historically been called biphenotypic, bilineal or mixed lineage leukemia. Acute leukemias of ambiguous lineage make up only a small percentage of acute leukemias in childhood. This group of leukemias all share the common feature of being very difficult to classify – either manifesting absent differentiation or expressing features of more than one lineage (B, T, or Myeloid). Due to its low incidence, decisions regarding treatments for acute leukemias of ambiguous lineage are very difficult to make and there is currently no accepted standard approach to treatment.

**Objectives:** To identify clinical and laboratory features at presentation, to document treatment protocol choice, and outcomes of acute leukemias of ambiguous lineage diagnosed at the Children's Hospital of Eastern Ontario, Canada.

**Design/Method:** All cases of acute leukemias of ambiguous lineage presenting to the Children's Hospital of Eastern Ontario from 2000 to 2014 were reviewed. Clinical features, laboratory data, treatment protocol utilized, adverse events and outcomes were examined.

**Results:** Four cases (2 male and 2 female patients between 6 and 15 years old) of acute leukemia of ambiguous lineage diagnosed since 2000 were identified and reviewed. All had similar clinical presentation, with variable severity of symptoms. Morphology and cytogenetic markers were variable. All had partial response to their initial induction treatment (3 AML and 1 ALL). The patient initially treated with ALL induction had the greatest response; he was the only patient with Mixed Phenotype T/Myeloid. All patients eventually went for bone marrow transplant, 2 are currently in long term follow up, 1 patient only recently underwent transplant, and 1 patient died shortly following transplant, she was the eldest patient.

**Conclusion:** Due to its rarity, there remains no standard consensus regarding therapy for leukemias of ambiguous lineage. Presentation of this case series contributes to the literature with the intention to expand our knowledge on the features of presentation and outcomes of treatment of this type of rare leukemia. This should ultimately help guide future management decisions of patients presenting with leukemia of ambiguous lineage.

Poster # 670

## **SEVERE RADIATION RECALL IN TISSUE AND LUNG AFTER HIGH DOSE STEREOTACTIC RADIOSURGERY IN A CHILD**

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**Background:** Radiation recall (RR) is a delayed inflammatory reaction throughout a previously irradiated area that is precipitated by the administration of certain drugs in the post radiation time frame. The severity of reaction varies based on dosage of radiation, timing and type of the inciting drug. The mechanism remains unknown. This reaction is very rare in children treated with radiation at standard doses. We report a severe case of radiation recall involving the skin and lung 6-9 months after high dose radiation therapy associated with doxorubicin.

**Objectives:** We report a case of an 8 year old child who presented to our institution with compartment syndrome and a large upper extremity osteosarcoma after receiving non-standard therapy of stereotactic radiosurgery (SRS) of 5 fractions of 800 rads delivered over 2 weeks. Radiation Oncology estimated the biologic equivalent dose as 200cGy x 45 fractions over 8 – 9 weeks or approximately 9000 Gy. Acutely the child had intense pain, edema, and erythema that responded to steroids. After the inflammation had subsided, the child received standard osteosarcoma chemotherapy of methotrexate, cis-platinum, doxorubicin, ifosfamide and etoposide with all the doxorubicin doses delayed 6 months in the hopes of mitigating RR. The patient achieved remission, but had grade 4 full thickness skin necrosis and ulceration with severe pain and fevers, 6 months from radiation with the first doxorubicin dose and then with subsequent doses (figure 1). The child had pain and fever relief with steroids, but poor wound healing and eventually needed an amputation. Full remission for two years.

**Design/Method:** We searched Google scholar, PubMed, and Ovid for similar cases of severe radiation recall in a child and for radiation recall of the lung.

**Results:** We did not find any similar cases in the last 40 years.

**Conclusion:** Radiation recall is an extremely rare but serious condition related to high dose, intensive radiation often exacerbated by inciting drugs such as doxorubicin in which the tissues recapitulate the injuries suffered during the initial dosing. Further research is needed to better understand this devastating side effect.

Poster # 671

## **SINUSIODAL OBSTRUCTION SYNDROME FOLLOWING LOW-DOSE 6-MERCAPTOPURINE THERAPY**

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**Background:** Juvenile myelomonocytic leukemia (JMML) is a rare myeloproliferative syndrome characterized by overproduction of monocytic cells. Sinusoidal obstruction syndrome (SOS) is thought to be caused by damage to hepatic endothelial cells from high-dose chemotherapy and/or ionizing radiation. It occurs most frequently during hematopoietic stem cell transplant (SCT) and is characterized by elevated bilirubin, hepatomegaly with right upper quadrant pain and weight gain.

**Objectives:** To describe the development of SOS in a patient with JMML treated with only low-dose 6-mercaptopurine (6-MP).

**Design/Method:** A 6-month-old male presented with fever and splenomegaly. White blood cell count was 60,000/ $\mu$ L comprised predominately of monocytes and myeloid precursor cells. Further testing lead to a diagnosis of JMML with both Monosomy 7 and a somatic KRAS mutation (G12V). His splenomegaly caused a consumptive coagulopathy and decreased pulmonary reserve, so low-dose 6-MP (50 mg/m<sup>2</sup>/day) was initiated for symptomatic

control. The patient had mild peripheral edema prior to therapy which dramatically worsened in the ensuing 5 days despite aggressive diuresis. His serum bilirubin rose from 2.1 to 16 mg/dL, prompting concerns for SOS. 6-MP was discontinued and a hepatic ultrasound with a Doppler flow study revealed increased resistance in hepatic arterial flow. His worsening coagulopathy was difficult to control, preventing the initiation of Defibrotide. Although his splenomegaly and coagulopathy worsened, his leukocytosis improved with a 5 day course of low-dose cytarabine (40 mg/m<sup>2</sup>/day). A liver biopsy and splenectomy were then performed. His consumptive coagulopathy improved after splenectomy. Histopathology of the spleen revealed myeloid precursors effacing the splenic architecture. The liver biopsy showed typical features of early SOS including central lobular congestion, hemorrhage, and hepatocyte ischemic necrosis.

**Results:** Hepatic function recovered and SOS resolved with aggressive supportive care. No further 6-MP was given. His disease progressed to AML, and he tolerated more intensive therapy with high-dose cytarabine, daunorubicin and etoposide. He has also tolerated a myeloablative SCT preparative regimen with busulfan, fludarabine and antithymocyte globulin.

**Conclusion:** SOS is infrequently seen outside of SCT and a literature review revealed no previous cases of documented SOS caused by low-dose 6-MP alone. The occurrence with low-dose therapy and spontaneous resolution suggests his massive splenomegaly contributed to the development of this condition.

Poster # 672

## A NOVEL MUTATION IN THE HARS GENE IN A PATIENT WITH INFANTILE MYOFIBROMATOSIS

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**Background:** An African American male presented to the emergency department at 53 days old with vomiting and nodules on his left posterior back near the left scapula, forehead, forearms and neck. All of the nodules were 1-2 centimeters in total dimensions, firm, without fluctuance or induration. CT of the brain showed 2 adjacent right frontal bone lucent lesions with a soft tissue component, which extended intracranially and extracranially. CT of the chest abdomen and pelvis showed myriad intramuscular soft tissue lesions and lytic lesions within the spine, pelvis and two pleural nodules in the right upper lobe of the lung. CT of the cervical and lumbar spine showed multiple lytic lesions within the spine and scattered throughout the musculature. Skeletal survey revealed innumerable lytic lesions in multiple bones. An excisional biopsy was performed and showed a nodular lesion composed of bland spindle cells with occasional mitoses. The cells were positive for smooth muscle actin with focal staining for muscle specific actin and desmin and negative for pancytokeratin, CD34, S100 and MART1, were consistent with intramuscular myofibroma consistent with infantile myofibromatosis (IMF). Given that the patient had no visceral lesions, patient was initially observed closely. However, he had an increase in the number and size of his fibromas and developed an Erb's Palsy so chemotherapy was initiated weekly with methotrexate and vinblastine.

**Objectives:** To determine the genetic abnormalities in the patient which might explain development of this condition.

**Design/Method:** Exome sequencing was performed from peripheral blood of the patient and unaffected parents.

**Results:** There were no mutations in the two known genes associated with infantile

myofibromatosis: PDGFRB and NOTCH3. There was a *de novo* mutation in HARS, which is a histidyl-tRNA synthetase, responsible for synthesis of histidyl-transfer RNA essential for incorporation of histidine into proteins. The gene product is frequently a target of autoantibodies in polymyositis and dermatomyositis as well as inherited peripheral neuropathies.

**Conclusion:** The HARS gene is a novel mutation in infantile myofibromatosis that may help explain gaps in knowledge of the genetic etiology.

Poster # 673

## **T-CELL ACUTE LYMPHOBLASTIC LEUKEMIA AND HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS DIAGNOSED AT PRESENTATION IN A 15 YEAR OLD MALE**

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**Background:** Hemophagocytic lymphohistiocytosis (HLH) is a life threatening disease of immune activation that can occur secondary to infections, rheumatologic diseases and malignancies. T-cell acute lymphoblastic leukemia (T-Cell ALL), an already high risk leukemia, is rarely reported as a pre-therapy diagnosis with HLH and poses challenging treatment hurdles.

**Objectives:** To report a concurrent diagnosis of T-Cell ALL and HLH and discuss a successful hybrid therapy used for treatment.

**Design/Method:** The patient is a 15 yo male who presented to our ER with multiple enlarged cervical lymph nodes, fever and fatigue. Laboratory evaluations indicated a leukopenia and thrombocytopenia. Upon peripheral smear and bone marrow studies, T-cell acute lymphoblastic leukemia was diagnosed. In addition, he met six criteria for HLH: fever, pancytopenia, splenomegaly, absent NK cell function, hyperferritinemia, hemophagocytosis on bone marrow review, and elevated sIL2R-alpha. He underwent treatment with cytarabine(IT), daunorubicin, etoposide, PEG-asparaginase, methotrexate(IT), dexamethasone and vincristine. After induction and consolidation he received a bone marrow transplant using total body irradiation, alemtuzumab, etoposide and cyclophosphamide conditioning.

**Results:** Initial laboratory workup revealed white blood cell count of 0.7 k/uL and platelet count of 23 k/uL. His ferritin was 5116 ng/mL, soluble IL-2R alpha was 10298 unit/mL and NK cell function was absent. After undergoing unrelated matched donor BMT, he experienced significant neuropathic pain, CMV pneumonitis and developed chronic kidney disease. Ultimately, these complications resolved, and he now has no evidence of disease 2 years since his original diagnosis.

**Conclusion:** We present this case to highlight an extremely rare pre-therapy diagnosis of HLH in the setting of T-cell ALL. An already high-risk leukemia, the concurrent diagnosis of HLH tremendously increases mortality if left untreated. We describe the criteria met for diagnosis and the medications used for treating this patient successfully. In addition, the treatment complications and their resolution are discussed. This is in contrast to previous reported cases in which T-Cell ALL with HLH had nearly 100% associated mortality. Most importantly, with this case, we hope to provide a successful example to an already difficult treatment regimen.

Poster # 674



## NEUROBLASTOMA PRESENTS AS CHRONIC DIARRHEA IN A PEDIATRIC PATIENT

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**Background:** Neuroendocrine tumors have a wide spectrum of presentation. A vasoactive intestinal peptide (VIP) secreting tumor is a rare form of a neuroendocrine tumor that primarily presents in the pancreas of adults. However, in children, they are more likely to present in the adrenal glands and arise from the sympathetic ganglia.

**Objectives:** To discuss the presentation and diagnosis of a VIP-secreting tumor whose pathology was consistent with stage III neuroblastoma in a pediatric patient.

**Design/Method:** Retrospective medical chart review.

**Results:** A two year-old male presented with six months of chronic diarrhea, subjective weight loss, abdominal pain with distention, and hypokalemia. Stool studies (electrolytes and infectious cultures), antibodies for celiac disease, and duodenal biopsies were all unrevealing. Initial abdominal ultrasound was interpreted as normal. He was thought to have “toddler’s diarrhea” and also treated with antibiotics for possible bacterial overgrowth. He returned eleven days later with new-onset vomiting with persistent hypokalemia, metabolic acidosis, and abdominal distention. Laboratory analysis revealed significantly elevated levels of gastrin, chromogranin A, and vasoactive intestinal peptide (VIP). CT scans revealed a large pelvic mass, and biopsy was consistent with neuroblastoma (Schwannian stroma-poor, >5% differentiating neuroblasts, low MKI, favorable histology). His urine catecholamines revealed a normal VMA and an elevated HVA. His neuroblastoma was classified as stage III as his tumor crosses midline, but his bilateral bone marrow evaluation and MIBG scan were negative in addition to his MCYN not being amplified. He was begun on octreotide to lower his level of VIP which improved his diarrhea. He was started on a chemotherapy regimen for intermediate-risk neuroblastoma (COG ANBL0531).

**Conclusion:** This patient’s symptoms responded initially to fluid and electrolyte replacement, in addition to dietary modification, which made early recognition of his diagnosis difficult. These rare tumors, affecting 1 in 10,000,000 people per year, must be treated aggressively. Thus, ongoing education of physicians is crucial. Diagnosis involves the identification of a hypersecreted hormone and the localization of a tumor by imaging. This class of neuroendocrine tumors should be considered in individuals with unexplained, high volumes of secretory diarrhea, electrolyte abnormalities, and elevated hormonal levels.

Poster # 675

## F317i MUTATION-ASSOCIATED NILOTINIB RESISTANCE IN A CHILD WITH CML; A FIRST REPORT

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**Background:** Philadelphia chromosome positive (Ph+) chronic myelogenous leukemia (CML) accounts for only 2-2.5% of childhood leukemia. Tyrosine kinase inhibitors (TKIs) have improved the prognosis of CML in adults and children. Failure of TKIs is often due to mutations. In adult CML, the F317i mutation has been implicated in resistance to first and second line



of identifiable feeding vessels while surgical resection was not an option due to multiple locations, high risk of anesthesia (prolonged QT and history of atrial fibrillation) and high probability of recurrence.

**Results:** Starting dose of Sirolimus was 0.8 mg/m<sup>2</sup>/dose twice daily. Dose was titrated to a target serum concentration of 5-10 ng/ml. However, our patient was exquisitely sensitive to Sirolimus and therapeutic levels were achieved at a dose of 0.24 mg/m<sup>2</sup>/dose. Pain resolved and patient was able to ambulate freely with partial radiographic improvement of the hamartomas associated with the AVMs. The therapy was well tolerated with hypercholesterolemia (elevated TG and LDL cholesterol) as the only noted side effect. However, after an initial improvement of 11 months patient developed pain in the left lower extremity. MRI revealed a bone infarct in the upper tibia at the location of the new pain. This adverse event is presumed to be a side effect of sirolimus.

**Conclusion:** Our case is unusual in that we achieved a symptomatic pain remission with a much lower dose of sirolimus than that published in literature (0.1mg/kg/day or 0.8 mg/m<sup>2</sup>/dose). This suggests the possibility of an altered metabolism unique to our patient. Due to rarity of these conditions multi-center studies are needed to determine optimal dosing and monitor for adverse effects of sirolimus in patients with PTEN hamartoma syndromes.

Poster # 677

## **AN UNUSUAL CASE OF EPSTEIN-BARR VIRUS (EBV) MASQUERADING AS LYMPHOMA IN A 16 YEAR OLD YOUNG MAN: A CASE REPORT**

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**Background:** EBV is a ubiquitous B-lymphotrophic herpesvirus infecting atleast 95% of population. Infection is usually self-limited, but has been implicated in the pathogenesis of several neoplastic and non-neoplastic disorders.

**Objectives:** Report an unusual presentation of EBV

**Design/Method:** Review of clinical and laboratory data

**Results:** A 16-year-old male presented with 7 days of fevers (tmax 104.7), cough, chills and night sweats. He reported significant mold at home and denied weight loss. A chest CT done for these symptoms showed multiple bilateral pulmonary nodules (few millimeters to 10 mm) involving both lungs, bulky mediastinal adenopathy (enlarged hilar, periaortic, subcarinal lymph nodes) and left supraclavicular lymphadenopathy. Abdominal CT was negative and no additional adenopathy or hepatosplenomegaly appreciated. He was transferred for concerns of malignancy. Testing revealed low platelet count (96,000/mcl), atypical lymphocytes on peripheral smear, elevated transaminases (AST/ALT: 263/293 units/L) and high LDH (1574 units/L). Diagnostic bilateral bone marrow aspirates, biopsies and excisional biopsy of the supraclavicular node were done. Lymph node pathology showed reactive lymphoid hyperplasia and *in situ* hybridization stain for EBV (EBER) demonstrated numerous positive cells consistent with EBV infection. Mixed inflammatory infiltrate with occasional large Hodgkin-like cells without unequivocal Reed-Sternberg cells were seen. Bone marrow biopsy showed no morphologic or immunophenotypic evidence of malignancy. EBV serology showed positive viral capsid IgM antibody, negative viral capsid IgG and EBNA antibodies, consistent with current active EBV infection. CMV IgM/IgG, Toxoplasma IgM/IgG, T. spot TB assay, beta-D-glucan assays and screens for HIV, Histoplasma and HHV8 testing (Castleman's disease) were all negative.

**Conclusion:** Diffuse pulmonary nodules is an unusual manifestation of EBV. This presentation is similar to lymphomatoid granulomatosis, a rare EBV associated systemic angiodestructive lymphoproliferative disorder that may progress to diffuse large B cell lymphoma, characterized by a polymorphous inflammatory milieu of small lymphocytes, plasma cells, histiocytes, with vascular infiltration, appreciable numbers of CD3+ T-cells, and variable numbers of CD20-positive B cells showing EBER positivity by *in situ* hybridization.<sup>1</sup> Our patient demonstrated EBER positivity in numerous cells, without a marked inflammatory infiltrate and immunohistochemical stains for CD20 and CD3 highlighted B and T lymphocytes in appropriate compartments. Follow-up for malignancy is warranted.

Poster # 678

## COMPLETE RESOLUTION OF VOCAL CORD PARALYSIS AFTER MEDULLOBLASTOMA TREATMENT

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**Background:** Vincristine (VCR) is a Vinca Alkaloid chemotherapy commonly used in treatment of childhood malignancies. Neurotoxicity following VCR is well documented, though the exact pathogenicity is not well understood. One of the probable mechanisms of neurotoxicity is that VCR interferes with microtubule formation through binding to  $\alpha$ -tubulin and disrupts axonal transport, in addition to its direct effect on conductance. VCR has been shown to cause primary axonal degeneration.

**Objectives:** To report a case of complete resolution of vocal cord paralysis after completion of VCR treatment without dose modification.

**Design/Method:** Case report and Literature review

**Results:** A seven-year-old male presented with nausea, vomiting, and ataxia. Brain MRI done with and without contrast showed a 5x5x4 cm mass in the 4<sup>th</sup> ventricle and hydrocephalus. He was diagnosed with average risk medulloblastoma. Patient underwent total resection of the tumor, radiation therapy, and chemotherapy according to COG protocol ACNS0331 that includes Vincristine, Cisplatin, Lomustine, and Cyclophosphamide. The patient developed progressive biphasic stridor noted after cycle 3. This stridor was persistent and worsened during the night. Flexible laryngoscopy showed bilateral vocal cord paralysis, and his swallow study was normal. There were no other signs of neuropathy. The decision was made to continue his chemotherapy regimen and attempt supportive care. His symptoms partially responded to Neurontin. Vincristine was continued at a full dose for the entire chemotherapy cycles (totaling 42 mg/m<sup>2</sup>). The patient had complete resolution of stridor within the 6 months after completion of therapy. He is 14 months off therapy with no stridor and with cessation of his Neurontin therapy within 4 months of completion of chemotherapy

**Conclusion:** Neuropathy is a well-known side effect of VCR. Isolated vocal cord paralysis is rarely reported in the literature. Most reported cases responded to cordectomy or treatment interruption. Our patient received continued treatment with Vincristine and symptomatic monitoring while treating with Neurontin. He experienced complete resolution of his stridor following completion of his therapy.

Poster # 679

# **FOCAL SEGMENTAL GLOMERULOSCLEROSIS IN ASSOCIATION WITH HODGKIN LYMPHOMA WITH COMPLETE RENAL IMPROVEMENT ON CHEMOTHERAPY: CASE REPORT AND LITERATURE REVIEW**

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**Background:** Nephrotic syndrome (NS) predating or occurring simultaneously with Hodgkin lymphoma (HL) is a known association with a reported incidence of 0.5-1%. Minimal change disease (MCD) is the most common glomerular pathology associated with HL. Focal segmental glomerulosclerosis (FSGS), however, is a rare association with less than 10 case reports in the adult and pediatric literature.

**Objectives:** To describe a case of HL presenting with FSGS at diagnosis.

**Design/Method:** Case report and literature review.

**Results:** A 14-year-old female presented with a two month history of periorbital and lower extremity edema which progressed to anasarca. She also presented with hypertension that was treated with an angiotensin-converting enzyme inhibitor (ACE-I). Family history was significant for maternal history of HL but not NS. Laboratory evaluation revealed a mild leukocytosis, high erythrocyte sedimentation rate, hypoalbuminemia, normal serum creatinine, microscopic hematuria, and nephrotic-range proteinuria (urine protein/creatinine ratio (Up/c) of 11.36). Imaging studies demonstrated a large paratracheal, hilar and paraspinal mass extending into the anterior mediastinum and cervical and supraclavicular adenopathy. Lymph node biopsy showed classical HL, lymphocyte-rich subtype. Renal biopsy showed FSGS. Additional evaluation included normal C3, C4 levels, and negative C- and P-antineutrophilic cytoplasmic antibody and lupus testing. Given stage-IIA HL (intermediate-risk), she was started on chemotherapy with doxorubicin, bleomycin, vincristine, etoposide, prednisone and cyclophosphamide. Hypoalbuminemia and microscopic hematuria resolved following one cycle of chemotherapy. Few weeks after, she was taken off ACE-I and continues to be normotensive. At completion of chemotherapy and while off the ACE-I for 2 months, the proteinuria fully resolved (Up/c: 0.07). F18-FDG/PET scan showed treatment response with residual mild FDG uptake and she is currently undergoing radiation therapy.

**Conclusion:** We describe a case of FSGS noted prior to or at the time of HL diagnosis/recurrence. Pathogenesis of MCD and FSGS in HL is poorly understood. Altered T-cell response causing cytokine-induced altered glomerular permeability has been implicated. Lymphoma therapy results in complete renal improvement, however, HL recurrence maybe associated with NS recurrence necessitating continued long term surveillance. In older children presenting with NS and a family history of HL, a thorough evaluation for underlying hematologic malignancies should be considered.

Poster # 680

## **USE OF mTOR INHIBITOR IN THE TREATMENT OF SUB EPENDYMAL GIANT CELL ASTROCYTOMA IN A PATIENT WITHOUT TUBEROUS SCLEROSIS**

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**Background:** Sub ependymal giant cell astrocytoma (SEGA) is a rare tumor most commonly seen in patients with tuberous sclerosis complex (TSC). SEGAs are slow growing ganglioneuronal tumors often located near the foramen of Munro and presenting with obstructive hydrocephalus. Everolimus, a mammalian target of rapamycin (mTOR) inhibitor, has been demonstrated to induce regression of SEGAs in patients with TSC.

**Objectives:** We sought to determine whether everolimus might also induce regression in a patient with SEGAs who did not have TSC.

**Design/Method (Case Study):** A 7 year old African American male presented with early morning headaches for 3 weeks and vomiting for 1 week. CT scan showed a 3.6x3.1 cm extra axial ventricular mass causing obstructive hydrocephalus. A gross total resection of the mass was achieved via trans-hemispheric approach. Pathology revealed giant cells, perivascular malformations, positive staining with GFAP and a small number of synaptophysin labeling cells consistent with SEGAs. He had no clinical stigmata of TSC and genetic testing for TSC1 and TSC2 gene mutations were negative. Three months post-operative, the patient again developed headaches. MRI revealed recurrent tumor. He was started on everolimus. After 2 months headaches resolved and after 6 months no residual lesion could be identified on MRI scan. Therapy was discontinued and after 1 year of follow-up no recurrence has been observed. Therapy was complicated by an acniform facial rash and vivid nightmares improving with dose reduction.

**Results:** The mTOR signaling pathway is involved in regulation of cell growth and proliferation and in inhibition of apoptosis. TSC1 and 2 gene products act as inhibitors of mTOR and their absence results in its constitutive activation. This is thought to contribute to development of hamartomas and tumors including SEGAs in patients with TSC. Our patient did not have TSC but responded well to everolimus, suggesting a similar pathogenesis. Molecular genetic testing is pending to determine if TSC 1 or 2 genes mutations are present in the tumor.

**Conclusion:** mTOR inhibitors may be useful in the treatment of SEGAs in patients without TSC; however, given the rarity of this tumor, multicenter trials will be required to determine safety and efficacy.

Poster # 681

## **EBV-NEGATIVE DONOR-DERIVED POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD) DEVELOPING 5 YEARS AFTER CORD BLOOD TRANSPLANT.**

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**Background:** Post-transplant lymphoproliferative disorder (PTLD) is believed to result from uncontrolled proliferation of lymphocytes in the setting of immune dysfunction. PTLD after hematopoietic stem cell transplant (HSCT) is uncommon with cumulative incidence ~1%, but has a high mortality. PTLD is usually Epstein Barr Virus (EBV)-positive.

**Objectives:** We present a rare case of EBV-negative PTLD. A 15 year old boy with history of adrenoleukodystrophy and a 6/6 HLA-matched single cord blood transplant in 2008 following busulfan-cyclophosphamide-horse anti-thymocyte globulin conditioning, cyclosporine and mycophenolate mofetil were used for graft versus host disease (GVHD) prophylaxis. He developed abdominal pain and fever and was diagnosed with perforated appendicitis in July 2013. He was fully engrafted and off of immunosuppressants since October 2010, although his

immune function was slow to recover. He was treated with antibiotics in July, and underwent appendectomy and draining lymph node biopsy in September 2013. Pathological evaluation of lymph nodes revealed EBV-negative monomorphic PTLD characterized as diffuse large B-cell lymphoma (DLBCL) type and non-germinal center subtype. Chimerism studies showed the lymphoma cells to be originating from donor cells. Disease extent workup revealed diffuse thickening of bowel wall with mesenteric lymphadenopathy on CT scan, negative PET scan, elevated LDH, normal bone marrow biopsy, and normal CSF. He was started on chemotherapy based on COG study protocol ANHL-0221 with rituximab, cyclophosphamide, and prednisone. Therapy complications included bowel perforation after the first cycle, which was treated with right hemi colectomy and end-ileostomy, and resumption of chemotherapy.

**Design/Method:** Clinical history and pertinent studies were obtained from medical records and literature review of similar cases was performed.

**Results:** Patient is currently disease free and discharged home at the completion of chemotherapy.

**Conclusion:** This is a unique case of EBV-negative PTLD from donor lymphocytes with distinct monomorphic DLBCL-like phenotype. EBV-negative PTLD after HSCT poses a specific challenge to clinicians because of its rare occurrence and lack of consensus about treatment.

Poster # 682

## **BRAIN TUMORS IN CHILDREN WITH SICKLE CELL DISEASE: TREATMENT CHALLENGES FOR YOUNG CHILDREN WITH CNS TUMORS AT RISK FOR CEREBRAL VASCULOPATHY**

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**Background:** Survival from pediatric CNS tumors is best achieved through maximal surgical resection, cranial radiation, or multiagent chemotherapy. Potential adverse outcomes include neurocognitive deficits, secondary neoplasms, vasculopathies, and cerebrovascular events (CVE). Individuals at increased risk may be those with an underlying alteration in cerebral vasculature or hematologic disease.

**Objectives:** To report on 2 novel cases of CNS tumors in patients with sickle cell disease (SCD) and considerations to minimize potential treatment-related neurologic sequelae.

**Design/Method:** Case 1: A 3-year-old male with HbSS and congenital left eye ptosis presented with progressive, daily emesis. MRI revealed nonmetastatic heterogeneous posterior fossa mass. Surgical resection left possible residual tumor along the medulla and pathology identified anaplastic ependymoma, WHO grade III. Treatment was initiated with focal proton beam irradiation without adjuvant chemotherapy, with prolonged partial exchange transfusions to minimize stroke risk.

Case 2: A 6-month-old male with HbSC presented with macrocephaly and hydrocephalus. MRI demonstrated a hypothalamic/chiasmatic glioma with intracranial metastasis and diffuse white matter abnormality. Pathology revealed pilocytic astrocytoma. Chemotherapy was initiated via COG9952. In both cases, therapies were considered with a focus on minimizing the risk of adverse CVE.

**Results:** Literature review and expert discussion revealed no previous cases of ependymoma and very few other CNS malignancies in SCD. Routine post-surgical radiotherapy for CNS tumors carries the potential for neurotoxicity including CVE. Baseline risk of CVE in patients with

HbSS is 1% with transcranial Doppler (TCD) screening. Younger patients and those with conditions that predispose to vasculopathy (like SCD) are more susceptible to irradiation-induced injury, including moyamoya collaterals and stroke. Chemotherapy to delay or avoid radiation is unproven in malignant ependymoma and risks renal disease, which accounts for 16% of deaths in adults with SCD. Prolonged partial exchange transfusions may minimize risks if successful in maintaining HbS levels of 30% or less. Case 1 developed post-radiation brain stem injury characterized by pontomedullary microhemorrhages, which is improving on steroids.

**Conclusion:** Treatment of CNS tumors requires balancing benefits/risks of multi-modal therapies. These unique cases highlight treatment complexities for malignant brain tumors in patients predisposed to cerebral vasculopathy and need for further therapeutic alternatives.

Poster # 683

## **MEDIASTINAL GRAY ZONE LYMPHOMA: AN UNCOMMON PEDIATRIC ENTITY**

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**Background:** Mediastinal Gray-Zone Lymphoma (MGZL) is a recently identified entity, with characteristics between primary mediastinal B-cell lymphoma (PMBL) and nodular sclerosis Hodgkin lymphoma (NSHL). Distinguishing features and treatment of MGZL are poorly defined in pediatrics. Traditionally, it has been treated with Hodgkin-like regimens with preplanned radiation. However, given that CD20 is expressed by most MGZLs, recent studies suggest that MGZL can be treated with rituximab using a dose intensified schema sparing radiation for salvage therapy.

**Objectives:** We report a 11-year-old African-American female who presented with 2 weeks of epigastric pain and decreased appetite. Family denied fever, night sweats, or weight loss. PET/CT scan of whole body revealed bulky mediastinal lymphadenopathy (max SUV of 11.4), moderate pericardial and pleural effusion with parenchymal lung nodules and tracheal compression. She underwent a mediastinal lymph node biopsy with results that showed large atypical Hodgkin cells but with strong and diffuse expression of CD20, PAX-5, MUM-1 and CD30, and weak and inconsistent expression of BCL-2, EMA, and CD15. Final pathology was diagnostic of B-cell lymphoma with features between diffuse large B-cell lymphoma and classical Hodgkin lymphoma. Cerebrospinal fluid was negative but bone marrow aspirate and biopsies showed 40% involvement on one side. She was diagnosed with Stage IV disease.

**Design/Method:** Based on the encouraging results of a recent prospective study involving largely adult patients with MGZL, patient was started treatment with infusional dose-adjusted etoposide, doxorubicin, and cyclophosphamide with vincristine, prednisone, and rituximab (DA-EPOCH-R) every 21 days supported with PEG-filgrastim. The patient tolerated the dose escalation, reaching ANC nadir < 500 after cycle 4 and mild mucositis. The patient is currently receiving her last cycle.

**Results:** Follow-up PET/CT after 4 cycles showed favorable response with resolution of nodes in the neck except for 2 PET avid nodes in the mediastinum and peri-gastric region (Max SUV 4.3).

**Conclusion:** MGZL is extremely rare in pediatrics and no cases reported with bone marrow involvement. Our approach using DA-R-EPOCH aims to avoid mediastinal radiation and its associated long term effects in this rare entity of non-Hodgkin's lymphoma with poor prognosis.



## **EXTENDED BEVACIZUMAB AND IRINOTECAN THERAPY IN PEDIATRIC PILOMYXOID ASTROCYTOMA**

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**Background:** Chemotherapy has a prominent role in treating non-resectable low grade glioma (LGG), which can be life threatening due to location and therapy resistance. Pilomyxoid astrocytoma (PMA), previously classified as a subtype of juvenile pilocytic astrocytoma (JPA), is clinically more aggressive and has a poorer prognosis despite chemotherapy with standard agents. Bevacizumab, an anti-angiogenic humanized monoclonal antibody directed at vascular endothelial growth factor-A, and Irinotecan, a topoisomerase I inhibitor, have shown some safety and efficacy in treating gliomas in adults and children. However, data is sparse regarding pilomyxoid astrocytomas and prolonged treatment duration in very young children.

**Objectives:** To describe efficacy of prolonged treatment with Bevacizumab and Irinotecan in pediatric PMA.

**Design/Method:** Retrospective review of patient medical records and literature review. Standard H&E and immunohistochemistry established the diagnoses. Patients were subsequently followed with serial physical, radiographic, and laboratory evaluations.

**Results:** Patient 1 presented at age 7 months with severe diencephalic syndrome and nystagmus. Patient 2 presented at age 20 months with irritability, near blindness, and diencephalic syndrome. In each child MRI showed a large, cystic, complex, homogeneously-enhancing suprasellar mass. Biopsy confirmed pilomyxoid astrocytoma WHO grade II. Patient 1 had progressive disease (PD) despite Carboplatin/Vincristine, monthly Temozolomide, and weekly Vinblastine. Bi-weekly Bevacizumab/Irinotecan infusions resulted in partial response (PR) then prolonged stable disease (SD) for 24 months, though 6 months after discontinuation he had PD. Bevacizumab/Irinotecan was restarted with PR then SD for 2 more years with no significant toxicity, though 6 months later he again had PD. Patient 2 had clinical and radiographic PD despite Carboplatin/Vincristine, and then Temozolomide, but rapid clinical and radiographic improvement upon initiation of bi-weekly Bevacizumab/Irinotecan. However after 1 year, due to perceived decline in vision, therapy was changed to Everolimus, though she developed clinical and radiographic PD after only 2 months. Re-initiation of Bevacizumab/Irinotecan resulted in immediate clinical and radiographic improvement and no significant toxicity.

**Conclusion:** Bevacizumab/Irinotecan represents a well-tolerated extended duration therapy option for children with progressive PMA despite treatment failure with conventional chemotherapy agents. This is particularly useful for younger patients needing prolonged therapy where delay of radiation is a primary goal.

## **PROLONGED COMPLETE RESPONSE IN A PEDIATRIC PATIENT WITH PRIMARY PERIPHERAL T-CELL LYMPHOMA OF THE CENTRAL NERVOUS SYSTEM**

Haneen Shalabi, Anne Angiolillo, Leigh Marcus

**Background:** Pediatric primary central nervous system lymphoma (PPCNSL) is an exceedingly rare malignancy accounting for 4% of all brain tumors, with only 2% of these classified as T-cell lineage. Most t-cell lymphomas in childhood have an immature phenotype, with a small percentage displaying mature differentiation. Despite aggressive treatment regimens with chemotherapy, whole brain radiation therapy (WBRT), and stem cell transplant, PPCNSL has been associated with a poor prognosis.

**Objectives:** We present a 12 year-old with PPCNSL, t-cell variant that achieved a complete remission (CR) when treated with a chemotherapeutic regimen used in adult CNS lymphoma.

**Design/Method:** Case Report

**Results:** Our patient presented with progressive headaches, right-sided weakness, blurry vision, and fatigue. CT scan demonstrated a left frontal mass. She underwent a complete resection and pathology was inconclusive. Her symptoms recurred one month later and MRI demonstrated recurrence of the left hemispheric mass with metastatic cerebral lesions. Biopsies showed abundant CD8 positive cells that were weakly positive for T-cell receptor gamma and partial loss of CD5. CD30 and ALK1 stains were negative; however PCR was positive for clonal T-cell receptor gene rearrangement, leading to the diagnosis of PPCNSL, t-cell variant. Metastatic work-up was negative and included: bone marrow assessment, lumbar puncture, and PET/CT. Two pediatric patients with primary CNS t-cell lymphoma have been reported; one patient was surgically resected and lost to follow-up, and the other died in the first cycle of chemotherapy. We initiated therapy with protocol CALGB 50202 used in adults with CNS lymphoma. Induction chemotherapy consisted of high-dose methotrexate (HDMTX) {8g/m<sup>2</sup> q 2 weeks} and temodar (TEM) {150mg/m<sup>2</sup> q 4 weeks}. After four months of therapy, MRI showed a CR. Consolidation therapy followed with 2 cycles of high-dose cytarabine (Ara-C) {2gm/m<sup>2</sup> q 12 hrs for 8 doses/cycle} and etoposide (ETOP) {40 mg/m<sup>2</sup> continuous over 96 hrs/cycle}. Our patient is 7 months off therapy, remains in CR, with a steadily improving neurologic exam.

**Conclusion:** PPCNSL, specifically mature t-cell lymphoma, is extremely rare in pediatric patients making a prospective therapeutic study very challenging. This case demonstrates that HDMTX-TEM-Ara-C-ETOP is a promising treatment option for pediatric patients with PPCNSL, thereby omitting detrimental side effects of WBRT.

Poster # 686

## **CRANIOPHARYNGIOMA PRESENTING AS ACUTE PSYCHOSIS: FIRST REPORTED CASE IN A PEDIATRIC PATIENT**

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**Background:** Craniopharyngiomas are benign, cystic epithelial tumors of the sellar or suprasellar region and are the most common nonglial pediatric brain tumor. Headache and visual field defects are the most common presenting clinical features in all age groups, followed by nausea/vomiting, delayed puberty, cranial nerve palsies and symptoms such as polyuria/polydipsia, hyperphagia, anorexia, and cognitive impairment.

**Objectives:** Describe the case of a 9-year-old female presenting with psychosis, auditory hallucinations and suicidal ideations found to have a craniopharyngioma. This patient also had a

5-month history of headaches, poor academic performance, and anorexia. She did not describe any changes in vision or feelings of fatigue. CT depicted a 3.9x4.1 cm hypodense mass arising from the suprasellar region with calcifications and hemorrhage. MRI studies showed a large, multilobulated, rim-enhancing, cystic suprasellar lesion consistent with a craniopharyngioma, confirmed by biopsy. The patient was treated with wide surgical resection and had complete resolution of her psychosis.

**Design/Method:** We conducted an extensive PubMed literature search, using the subject headings: brain neoplasms AND psychotic disorders, or brain neoplasms AND suicidal ideations. Article types were limited to case reports, and age was restricted to birth to 18 years old.

**Results:** Pediatric brain tumors presenting as acute psychosis are exceedingly rare, and craniopharyngiomas presenting with psychotic symptoms have never been previously described. We identified 15 unique published cases of intracranial neoplasms presenting as acute psychosis. 7 of these cases pertained to patients under 18 years old, and 2 of the 7 cases were not published in English. Therefore, 5 unique case studies revealed a similar presentation to that of our patient, however different tumor types were identified.

**Conclusion:** We believe this is the first case of acute psychosis as the main presenting symptom in a patient with a craniopharyngioma, both in the pediatric and adult population

Poster # 687

## **RARE CENTRAL NERVOUS SYSTEM INVOLVEMENT PRESENTING IN TWO PEDIATRIC PATIENTS WITH POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER PATHOLOGIC SUBTYPE PLASMABLASTIC LARGE B-CELL LYMPHOMA**

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**Background:** Post-transplant lymphoproliferative disorder (PTLD) presenting as plasmablastic large B-cell lymphoma (PLBCL) is rare. There are no reported pediatric cases of central nervous system (CNS) involvement. Two pediatric patients developed CNS involvement, leading to the demise of one patient.

**Objectives:** Determine prognostic factors and treatment for CNS involvement in PLBCL.

**Design/Method:** Patients were treated with known effective PLBCL therapy at reduced doses due to multi-organ transplant related toxicity. EPOCH-R (etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin, rituximab) and bortezomib, plus IT methotrexate were used for patient one, and R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, prednisone) with triple IT (methotrexate, cytosine arabinoside, hydrocortisone) and craniospinal radiation in the second.

**Results:** Patient one: 9-year-old male status-post small bowel transplant 4 years prior, chronic Epstein-Barr and norovirus, presented with obstructive abdominal tumor positive for CD45, CD138, C-MYC, EBER, MUM1, kappa light-chain restricted, Ki-67 90%. Bone marrow, MRI brain, and CSF cytology were negative, but complicated by CNS mycoplasma. On disease progression treated with alemtuzumab, vincristine, etoposide without response. Autopsy demonstrated lymphomatous meningitis, involvement over cranium and spinal cord, and diffuse dural nodules. Patient two: 14-year-old male with multi-visceral organ transplant 2 and 5 years prior, presented with a mass extruding from J-tube site, CD19, CD45, CD56, CD117, CD138,

EBER, MUM1 positive, kappa light chain restricted. Ki-67 85%. His CSF was 2a, bone marrow and brain MRI negative. 9 months prior he had plasma cell PTLT treated with steroids and bortezomib. Completed R-CHOP x 6 and triple IT twice weekly x 4 then monthly followed by craniospinal radiation.

**Conclusion:** PLBCL PTLT is extremely rare with no reported pediatric CNS cases and dismal outcomes. Aggressive chemotherapy often requires dose modification in multi-visceral organ transplants. Patient one was CNS negative at diagnosis; CNS involvement was found post-mortem despite IT therapy. Patient two was CNS 2a status. Although CNS involvement is rare in PLBCL, it should be considered in all patients with PLBCL PTLT, with the understanding that imaging and laboratory results may be negative, and symptoms may not be present. Thus craniospinal radiation with IT therapy for prophylaxis and treatment and IT rituximab for resistant disease could be considered.

Poster # 688

## **SUCCESSFUL USE OF PROTON BEAM THERAPY IN A 13-YEAR OLD MALE WITH BASAL GANGLIA GERMINOMA**

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**Background:** Basal ganglia germinomas comprise 5-10% of all intracranial germ cell tumors. Symptoms can vary leading to a delayed diagnosis. These tumors are highly responsive to chemotherapy and radiation; however, craniospinal radiation can lead to serious side effects. Alternatively, proton therapy is a more focused treatment that can be used to limit many of these complications.

**Objectives:** We present the case of a child with atypical findings of a germ cell tumor who received proton therapy instead of craniospinal radiation. By doing so, we hope to increase awareness of atypical presentations of intracranial germ cell tumors. Additionally, we report using proton therapy in the hopes of limiting long-term side effects from radiation.

**Design/Method:** A retrospective chart review was performed on a 13-year old male at Maimonides Medical Center, an urban community hospital in Brooklyn, New York. Local IRB approval and parental consent was obtained.

**Results:** The patient presented with two months of progressive difficulty walking, bradykinesia, rigidity of the right extremities, headaches, and difficulty speaking. MRI of the brain identified an extensive lesion in the left basal ganglia with evidence of supratentorial leptomeningeal dissemination. Alpha-fetoprotein was elevated at 118ng/ml (reference range <6.1ng/ml) and HCG was negative. A stereotactic biopsy confirmed diagnosis of basal ganglia germinoma. The patient had a near complete response to six cycles of induction chemotherapy followed by consolidative radiation therapy according to ACNS0122. Radiation therapy (RT) was delivered with uniform scanning proton beam therapy to the craniospinal axis (36 CGE) and a boost to the prechemotherapy extent of disease (18 CGE) for a total dose of 54 CGE. The patient tolerated treatment without interruptions. He is in remission 16 months after completion of therapy with minimal neurological deficits.

**Conclusion:** Primary CNS germ cell tumors need to be considered when a patient presents with a progressive constellation of neurological deficits to avoid delay in diagnosis and to ensure timely therapy. Although germinomas are highly sensitive to chemotherapy and radiation, the

risk of late effects of RT in children are substantial and the use of proton beam therapy may mitigate some of the late effects.

\*(Rasalkar et. al., Br J Radiol, 2010).

Poster # 689

## **THE FIRST AND SECOND GENERATION TYROSINE KINASE INHIBITORS ROLE IN THE PHILADELPHIA POSITIVE CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA**

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**Background:** Leukemia is the most common malignancy in childhood cancers. In recent years, studies focus on the identification of subgroups for the diagnosis and also targeted therapies for the excellent prognosis.

**Objectives:** The effectiveness of tyrosine kinase inhibitors (TKI) has proven in the Philadelphia chromosome positivity (Ph<sup>+</sup>) as known a very high-risk ALL. In this study, we reported our Ph<sup>+</sup> ALL patients.

**Material-Method:** Files of children in the Istanbul University, Oncology Institute were evaluated retrospectively for demographic data, treatment and outcome.

**Results:** During 2000-2014, 8 children (4 girls, 4 boys) with a median age of 7 (3,5-19) were diagnosed with Ph<sup>+</sup>ALL. Six of them were prekursor B.ALL, 2 of them T.ALL. At the initial diagnosis 4 of them were standart risk and 4 of them were high risk. Remission was achived 7 of the patients in the 15th day except one who had remission at 29th day. Six of the patients have Ph<sup>+</sup> at the diagnosis. In 1 patient's positivity was detected when she had combined relaps (conjunctiva+bone marrow) after 12 months from the cessation of the treatment. And the other one had a long treatment pause for TRALI (transfusion releated acute lung injury) . Because of this long period bone marrow aspiration was performed before the begining of the maintenance and Ph<sup>+</sup> was detected. When Ph<sup>+</sup> was reported, 4 of the patients were treated with imatinib and the other 4 with dasatinib. All of them had negativity in 2 weeks. One patient died because of enterococcal sepsis during the phase IV and 1 refuge patient went back to his country after remission. The others are all alive and still in remission.

**Discussion:** When Ph<sup>+</sup> was detected in the ALL patients, they are included in very high risk group. TKI's (1<sup>st</sup> and 2<sup>nd</sup> generation) are very effective at the treatment of this subgroup and also they can be used safely. However, the stem cell transplantation is challenging.

Poster # 690

## **CASE SERIES OF PEDIATRIC GLIOBLASTOMA MULTIFORME**

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**Background:** Glioblastoma multiforme (GBM) is a grade IV astrocytoma and is the most aggressive malignant primary brain tumour. The incidence of GBM is 2 to 3 per 100,000 people.

GBMs are uncommon in children, accounting for less than three percent of childhood brain tumours.

**Objectives:** This case series reviews the incidence, pattern of presenting symptoms, tumour location, patient treatment and overall survival of pediatric GBM patients.

**Design/Method:** The Children's Hospital of Eastern Ontario is a medium size program with 70 to 75 new diagnoses of malignancy each year. All cases of GBM for the past 2 years were reviewed and data collected.

**Results:** During the study period, 5 patients were diagnosed with primary *de novo* GBM and one with secondary GBM. The incidence rate was 5%. Four primary GBM were found in the cerebral hemispheres, most commonly affecting the right frontal lobe, one was in the brainstem and one in the spinal cord between C4 to T1. The secondary GBM was in the left parietal occipital lobe and was secondary to radiation for a primary lymphoma diagnosed 4 years previously. GBM occurred at a mean age of 9.46 years. The male to female ratio was 2:1. Main presenting symptoms included headaches (67%), vomiting (50%), nausea (33%) and tonic clonic seizures (33%). Treatment included radiation therapy for all patients and those that received 55.8 Gy in 31 fractions displayed the highest mean survival of 1 year and 3 months, whereas those who received 54 Gy to 59.4 Gy in 33 fractions survived a mean of 7 months. Both groups were treated with chemotherapy including temozolomide during and after radiation, and others agents included lomustine and bevacizumab. The two patients that received a gross complete resection survived an average of 7 months, and the three that received a partial resection survived an average of 8.5 months. One patient received a surgical biopsy and survived 1 year and 10 months. The median survival was 10.6 months.

**Conclusion:** The outcome of Glioblastoma multiforme at our institution is poor and incidence higher than expected; further study needs to investigate if this is a local or national occurrence.

Poster # 691

## EXERCISE PRACTICES, PREFERENCES, AND BARRIERS IN THE PEDIATRIC ONCOLOGY POPULATION

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**Background:** Childhood cancer survivors have increased risk of late effects including cardiac disease, obesity, subsequent cancers, metabolic syndrome, and psychological distress that could be mitigated by regular moderate physical activity.

**Objectives:** To determine exercise preferences, practices, and barriers among patients during and after therapy at a tertiary care referral center.

**Design/Method:** Cross-sectional study of English-speaking patients  $\geq 4$  years old attending the Yale Pediatric Oncology Clinic from October 2013 - October 2014 with a cancer diagnosed at  $\leq 21$  years. Participants were  $>1$  month since diagnosis, not acutely ill the day of visit, with a life expectancy of  $>6$  months. Participants (parents of those  $<18$  years) completed a 10-minute written survey and received a \$5 honorarium.

**Results:** Overall, 161 (99% eligible) participants were an average age of 15.6 years (range 4-53). Diagnoses included leukemia (43%), lymphoma (24%), CNS tumors (6%), solid tumors (21%), and other conditions (6%); 66.5% were off-therapy. Predictors of meeting CDC physical activity guidelines included being off therapy ( $p=0.008$ ) and age  $\geq 18$  years ( $p=0.001$ ). Treatment intensity ( $p=0.78$ ) and diagnosis ( $p=0.20$ ) were not associated with physical activity. Notably,

89% of participants preferred exercising at home and 73% preferred walking for exercise.

**Conclusion:** Most childhood cancer survivors did not meet the CDC weekly physical activity guidelines, especially if younger or on-therapy. Reported barriers and preferences can help guide intervention strategies to improve exercise behaviors in patients both on and off therapy.

Pediatric Oncology Patients' Exercise Practices, Preferences, and Barriers			
	Total n= 161	On Therapy n= 54	Off Therapy n= 107
Hours of Moderate/ Vigorous Activity per week:			
Overall	4.3 ± 4.1	3.9 ± 4.6	4.5 ± 3.9
4-11.9 years	5.3 ± 5.1	4.5 ± 5.2	5.9 ± 5.0
12- 17.9 years	4.1 ± 3.9	3.7 ± 4.7	4.4 ± 3.6
≥ 18 years	3.7 ± 3.2	3.5 ± 3.5	3.7 ± 3.1
Percent Meeting CDC Exercise Recommendations:			
Overall	35%	20%	42%
4-11.9 years	24%	14%	26%
12- 17.9 years	24%	14%	30%
≥ 18 years	59%	46%	63%
Preferred Exercise Location:			
Home	89%	89%	90%
Park	57%	63%	54%
Gym	63%	44%	73%
Clinic	19%	26%	16%
Hospital	13%	20%	9%
Preferred Exercise Activities:			
Walking	73%	78%	71%
Biking	62%	76%	55%
Swimming	44%	59%	37%
Jogging	35%	24%	40%
Barriers to Exercise:			
Fatigue	32%	46%	24%
Parental fear of injury	19%	35%	10%
Expense	18%	20%	17%
Afraid of injury	13%	20%	9%
Lack of injury	21%	15%	25%
Doctor never recommended	8%	9%	7%

Poster # 692

## A NOVEL APPROACH TO INVESTIGATING THE PATHOGENESIS OF ONCOGENIC TRANSLOCATIONS IN INFANT LEUKEMIA USING ENGINEERED NUCLEASES

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**Background:** One of the challenging questions in cancer biology is how a normal cell transforms into a cancer cell. Chromosomal translocations are a key element in this transformation process in a variety of pediatric cancers. For the majority of infants who develop acute lymphoblastic leukemia (ALL), a translocation of the *MLL* gene *in utero* serves as the initiating event for leukemogenesis. Clinically, the *MLL* translocation confers a particularly poor prognosis for infants. The mechanism by which this translocation contributes to treatment refractory disease remains poorly understood.

**Objectives:** To create a novel model of infant ALL using genome engineering of *MLL* translocations to prospectively examine the process of leukemogenesis and the mechanisms underlying the pathogenesis of *MLL-AF4* translocations.

**Design/Method:** Engineered nucleases were used to induce simultaneous double strand breaks in the *MLL* and *AF4* genes resulting in specific *MLL-AF4* and the reciprocal *AF4-MLL* translocations in primary hematopoietic stem and progenitor cells (HSPCs). These cells were monitored over time *in vitro* to assess for survival advantage and transformation potential as well as immunophenotype and gene expression changes induced by the *MLL* translocation. These

cells were also introduced into sub-lethally irradiated NSG mice to assess for engraftment and leukemic potential.

**Results:** Generation of a specific double strand break within the breakpoint cluster region of the *MLL* and *AF4* genes was sufficient to generate *MLL-AF4* translocations, as well as the reciprocal *AF4-MLL* translocation in primary HSPCs resulting in expression of the *MLL-AF4* fusion transcript. Sub-populations of HSPCs with the induced *MLL* translocation demonstrated a significant survival advantage *in vitro*, but the translocation alone was not sufficient for leukemic transformation. Gene expression analysis and *in vivo* analysis of the leukemic potential of these cells in NSG mice are on going.

**Conclusion:** These studies demonstrate the feasibility of using genome engineering to induce patient specific chromosomal translocations into primary human HSPCs to prospectively analyze the mechanism of translocation-associated leukemogenesis. Given the high proportion of pediatric cancers that result from chromosomal rearrangements, we believe this approach will be beneficial to more accurately investigate the mechanism of pathogenesis in a variety of pediatric cancers.

Poster # 693

## A COMPARISON OF ACUTE LYMPHOBLASTIC LEUKEMIA IN THE US AND MEXICO AT TWO SINGLE INSTITUTIONS

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**Background:** Acute lymphoblastic leukemia (ALL) is the most common childhood cancer with overall survival rates nearing 85% in many parts of the world. In the United States, Hispanic children, compared to other defined racial and ethnic groups, are noted to have a higher incidence of ALL and one of the poorest outcomes. Mexico has one of highest incidences of ALL in the world.

**Objectives:** To compare basic characteristics and clinical presentation of children diagnosed with ALL at Hospital del Niño Morelense (HNM) in Morelos, Mexico and Children's Mercy Hospital (CMH) in Kansas City, Missouri.

**Design/Method:** Retrospective clinical data including patient characteristics, clinical presentation, National Institute of Cancer (NCI) criteria, cytogenetic profiles and cause of mortality were collected from medical records at Hospital del Niño (n=62) and Children's Mercy Hospital (n=66) between January 2011 to July 2013. Only patients with de novo ALL were included. Descriptive statistics and Fisher's exact analysis were used to analyze data.

**Results:** All patients at HNM were Mexican and the majority of patients at CMH were Caucasian. Differences in immunophenotype (B versus T cell) were similar. The number of male patients was higher at HNM (69%) vs CMH 48% (p<0.008). Excluding T-cell ALL, the overall number of high risk patients per NCI criteria was similar. However the number of patients with WBC >50,000 was higher at HNM (21%) vs CMH (9%) (p=0.07). Criteria based upon age in years (<1 or >10) was similar. Central nervous system infiltration occurred in 9/62 (14.5%) of patients at HNM and 1/66 (1.5%) at CMH (p=0.007). There was an increased incidence at HNM of patients with 11q23 rearrangements (p=0.005) in non-infants, t(9;22) (p=0.011) and hypodiploidy compared to CMH. The mortality rate was 6% at CMH and 12.9%



at HNM ( $p=0.232$ ) among this group of patients.

**Conclusion:** There was a significant difference in the number of male patients with ALL at HNM compared to CMH. Patients at HNM in Morelos, Mexico demonstrate more high risk features as compared those at CMH. Future international collaboration is warranted to better understand leukemia globally.

Poster # 694

## **REGULATION OF PI3K PATHWAY BY IKAROS AND CASEIN KINASE II (CK2) IN PEDIATRIC LEUKEMIA**

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**Background:** *IKZF1* encodes a DNA-binding, zinc finger protein that regulates expression of genes involved in important biological pathways including phosphatidylinositol-3-Kinase (PI3K) pathway. Function of Ikaros is impaired in high-risk pediatric B-cell acute lymphoblastic leukemia (B-ALL). Chromatin immunoprecipitation coupled with next-generation sequencing (ChIP-SEQ) was used to show that Ikaros binds to the upstream regulatory regions of multiple genes that regulate the phosphatidylinositol-3-Kinase (PI3K) pathway. PIK3C2B and PI3KFYVE were among Ikaros target genes.

**Objectives:** 1) To determine how Ikaros regulates transcription of its target genes PIK3C2B and PI3KFYVE and affect PI3K Pathway. 2) We have previously shown that a pro-oncogenic Casein Kinase II (CK2) can directly phosphorylate Ikaros *in vivo* and that CK2-mediated phosphorylation impairs Ikaros function. To test whether inhibition of CK2 activity affects ability of Ikaros to regulate transcription of PIK3C2B and PI3KFYVE in leukemia.

**Design/Method:** Overexpression of Ikaros via retroviral transduction in Nalm6 cells, Transfection of Nalm6 cells with Ikaros shRNA, qRT-PCR, Luciferase reporter assay, quantitative chromatin immunoprecipitation (qChIP).

**Results:** Results from loss-of-function and gain-of-function experiments suggest that Ikaros functions as a transcriptional repressor of PIK3C2B and PI3KFYVE genes in leukemia. Molecular and pharmacological inhibition of CK2 showed similar effect on transcription of Ikaros target genes and they resulted in transcriptional repression of both PIK3C2B and PI3KFYVE genes. Treatment of leukemia cell lines, as well as primary B-ALL cells, with different CK2 inhibitors resulted in enhanced Ikaros binding to its target genes, as evidenced by qChIP.

**Conclusion:** Ikaros and CK2 regulate the PI3K pathway via transcriptional regulation of the PIK3C2B and PI3KFYVE genes. CK2 inhibition enhances Ikaros activity as a transcriptional repressor of genes that promote the PI3K pathway in primary B-ALL cells. CK2 inhibitors have potential to therapeutically restore Ikaros function in B-ALL and cause antileukemic effect.

Poster # 695

## **CORRELATION BETWEEN BONE MARROW SPICULARITY AND MINIMAL RESIDUAL DISEASE AMONG CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA**

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**Background:** Bone marrow (BM) spicularity is a widely acknowledged metric for sample quality, and may have important implications in measuring minimal residual disease (MRD) in leukemia patients undergoing therapy. When BM samples are aspicular, there may be a heightened chance for hemodilution and false negative MRD.

**Objective:** The objective of this study is to assess whether the presence or absence of spicules in the BM samples of patients with acute lymphoblastic leukemia (ALL) correlates with the detection of MRD at the end of induction therapy.

**Design/Method:** A retrospective chart review was performed for patients that were diagnosed with ALL between January 2010 and June 2014. The age range of these patients at the end of induction was 12 months to 22 years, and excluded infants (age <1). MRD was measured by flow cytometry (FC). Results were examined from our local lab and an external reference lab, which has greater FC sensitivity. MRD was considered positive if >0.01%.

**Results:** Preliminary findings (n=214) demonstrated a correlation between BM spicularity and negative MRD per our local lab analyses (p=0.019), but not by the reference labs (p=0.111). Moreover, there were 8 false negatives by local FC, all of which were equal or less than 0.1%, with most being <0.05% by the reference laboratory.

**Conclusion:** These findings suggest that aspicular BM samples may be associated with a higher percentage of negative MRD results. This correlation was statistically significant for local MRD results, but not for MRD from reference labs. The increased sensitivity of the reference laboratories may partially overcome the hemodilution in aspicular samples. These findings support using a laboratory with highly sensitive FC for MRD detection. We are currently investigating the correlation between spicularity and degree of MRD detected by FC, which will allow us to quantify this relationship.

Poster # 696

## **WEANED PIG MODEL OF ASPARAGINASE-INDUCED PANCREATITIS**

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**Background:** Asparaginase effectively treats acute lymphoblastic leukemia, but has toxicities that can limit its use. Thrombosis, hyperglycemia, and dyslipidemia are managed with medical therapy, but pancreatitis often requires premature cessation of asparaginase.

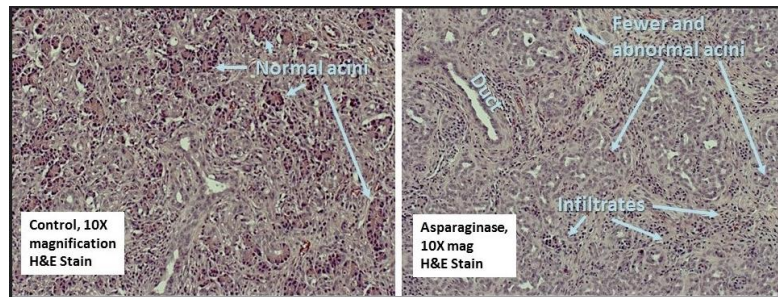
**Objective:** Establish a large-animal model of asparaginase-induced pancreatitis.

**Methods:** Ten weaned 3-week old female pigs from a genetically consistent background had jugular vein and re-entrant pancreatic duct/proximal duodenal catheters surgically placed then received 10 doses of Erwinia asparaginase administered at 48-hour intervals with serial sampling of blood for amylase and pancreatic secretions to assess volume and composition. Animals were observed for infusion reactions, weight gain, feeding, and behavior. After completion of asparaginase doses, animals were necropsied and organ histology reviewed. Four controls had catheters placed but received no asparaginase. Jazz Pharmaceuticals provided funding.

**Results:** The pig model proved feasible. All doses of asparaginase were administered, CVCs remained functional for the duration of the study, and pancreatic catheters provided useful data

for 1-2 weeks after placement without clinical complications. Pigs who received asparaginase had normal activity and feeding habits, but weight gain was minimal (mean 5.2kg ( $\pm 0.6$ SE) to 5.7 kg (10% increase) during the 3-week study, compared to controls that doubled their weight. Histology at necropsy was consistent with acute pancreatitis, and showed both ductal and acinar changes (dilatation, apoptosis, attenuation), as well as infiltrates (Figure).

**Conclusions:** The weaned pig model faithfully replicates many of the features of asparaginase toxicity observed in humans, including pancreatitis. Next steps will include identification of biomarkers, preventive strategies, and treatments for pancreatitis.



Poster # 697

## ACUTE NEUROLOGIC DETERIORATION IN CHILDREN ON CHEMOTHERAPY FOR LEUKEMIA - TIMING, ETIOLOGY AND OUTCOME

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**Background:** Various neurologic complications with similar presentation occur during the course of chemotherapy for leukemias in children. MRI and CSF analysis are important tools for diagnosis, and therapy differs widely, based on etiology.

**Objectives:** To present the timing, etiology and outcome of acute neurologic deterioration (focal neuro-deficit/ seizures/ encephalopathy) during chemotherapy for leukemia.

**Design/Method:** Retrospective study of all urgent neurology referrals for children on chemotherapy, from May2010- May2013.

**Results:** n =19. 13 events occurred during Induction and 6 during maintenance. 8 events were due to L-asparaginase (3 infarcts and 5 dural sinus thromboses), following the initial doses in induction phase. FFP was given acutely and with all further doses of L-asparaginase. 6 weeks' course of LMWH/Aspirin was given. 4 of them recovered without sequelae. Hypertensive-encephalopathy with characteristic FLAIR-MRI findings of PRES due to ITMTx was seen in 5 cases; treated with supportive measures and anti-hypertensives. Upon recovery, further doses were reduced and folate was supplemented. 5 children had viral encephalitis (MRI+CSF findings) and improved with acyclovir. 4 have sequelae. One child with left MCA territory infarct due to CNS relapse, expired in the acute phase. Outcome was normal in 10 children, who had either PRES or DVST. 4 children have residual hemiparesis. 2 children have epilepsy (both had EPC at presentation). 2 children have hemiparesis with epilepsy.

**Conclusion:** While survival rates for leukemias have improved, morbidity from neurologic sequelae hampers the quality of life. Active research for specific prophylactic therapy and less-toxic chemotherapeutic molecules will be important to reduce morbidity.

No.	Sex/Age M: Male F: Female	Type of malignancy	CNS status	Phase of chemo therapy	Focal deficit	MRI finding	EEG	Final diagnosis	Outcome of neurologic complication
1	F/ 10	B cell ALL	CNS1	Maintenance	Hemiparesis	Infarct in the Right fronto-parietal region	-	L Asp - Infarct	Residual paresis
2	F/ 10	B cell ALL	CNS1	Induction	Focal seizure with Hemiparesis with bladder incontinence	Infarct in the left MCA territory	Focal	L Asp - Infarct	Seizure disorder + Residual paresis
3	F/ 10	B cell ALL	CNS3	Induction	Focal seizure with Hemiparesis	DVST	Focal	L Asp - DVST	Residual paresis
4	M/ 7	B cell ALL	CNS2	Maintenance	GTCs with Hemiparesis with aphasia	Left MCA territory Infarct	EPC	L Asp - Infarct	Seizure disorder
5	F/ 4	B cell ALL	CNS1	Induction	Encephalopathy with raised ICP	DVST	-	L Asp - DVST	Normal
6	M/ 2	B cell ALL	CNS1	Induction	Encephalopathy with raised ICP	DVST	-	L Asp - DVST	Normal
7	M/ 11	T cell ALL	CNS1	Induction	Focal seizure	DVST	Focal	L Asp - DVST	Normal
8	F/ 3	B cell ALL	CNS1	Induction	Encephalopathy with raised ICP	DVST	-	L Asp - DVST	Normal
9	M/ 5	Pre B cell ALL	CNS3	Diagnosis of CNS Relapse	Focal seizure with Hemiparesis with RUL dystonia	Left MCA territory Infarct	EPC	Leukemic Infarct in CNS relapse	Expired
10	F/ 6	Pre B cell ALL	CNS2	Maintenance	Fever, Focal seizure with Hemiparesis	Cerebral edema in the Right frontal region	Focal	Viral Encephalitis on CSF	Normal
11	M/ 2	B cell ALL	CNS1	Maintenance	Fever, encephalopathy, Hemiparesis	White matter hyperintensities in the Occipital region	-	Viral Encephalitis on CSF	Residual paresis
12	M/ 4	Pre B cell ALL	CNS1	Induction in Testicular relapse	Fever, encephalopathy, Focal seizure with Hemiparesis	Right sub-cortical white matter hyperintensities	Focal	Viral Encephalitis on CSF	Seizure disorder + Residual paresis
13	F/ 8	T cell ALL	CNS1	Induction in Bone marrow relapse	Fever, encephalopathy, Focal seizure with Hemiparesis	Right temporal (Peri Rolandic) white-matter hyperintensities	EPC	Viral Encephalitis on CSF	Seizure disorder
14	M/ 8	Pre B cell ALL	CNS1	Maintenance	Fever, Focal seizure with Hemiparesis	Right Parieto-occipital region hyperintensities	Focal	Viral Encephalitis on CSF	Residual paresis
15	M/ 7	B cell ALL	CNS1	Maintenance	HTN, GTCs, aphasia, encephalopathy	FLAIR Parieto-occipital WM Hyperintensities	-	PRES	Normal
16	M/ 4	B cell ALL	CNS1	Induction	HTN, GTCs, encephalopathy	FLAIR Parieto-occipital WM Hyperintensities	-	PRES	Normal
17	M/ 9	B cell ALL	CNS1	Induction	HTN, GTCs, visual disturbance, encephalopathy	FLAIR Parieto-occipital WM Hyperintensities	-	PRES	Normal
18	F/ 13	Pre B cell ALL	CNS1	Induction	HTN, visual disturbance, GTCs, encephalopathy	FLAIR Parieto-occipital WM Hyperintensities	-	PRES	Normal
19	M/ 3	Pre B cell ALL	CNS1	Induction	HTN, GTCs, encephalopathy	FLAIR B/L dorsal pons WM Hyperintensities	-	PRES	Normal

ALL: Acute Lymphoblastic leukemia; B/L: Bilateral; CSF: Cerebro-Spinal Fluid; DVST: Dural Veous Sinus Thrombosis; EEG: Electro Encephalo -Gram; EPC: Epilepsia Partialis Continua; FLAIR: Fluid Attenuation Inverted Recovery Sequence MRI; GTCs: Generalized Tonic Clonic seizures; HTN: Hypertension; L-Asp: L-Asparaginase; PRES: Posterior Reversible (leuco) Encephalopathy Syndrome; WM: White-matter

Poster # 698

## THE WEANED PIG AS A MODEL FOR ASPARAGINASE INFUSION REACTIONS

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**Background:** Infusion reactions can complicate asparaginase administration and are caused by hypersensitivity and/or hyperammonemia.

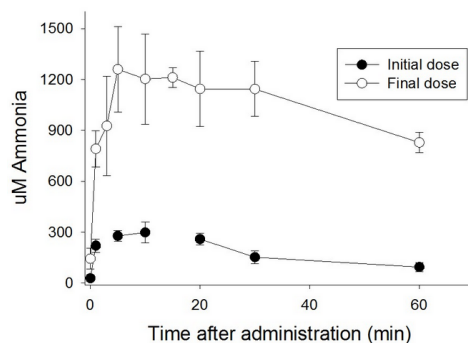
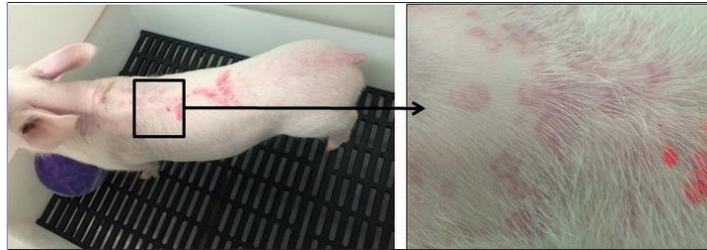
**Objective:** Develop a pig model for asparaginase infusion reactions.

**Methods:** Jugular catheters were surgically placed in 5 juvenile weaned pigs for administration of Erwinia asparaginase (human equivalent dose of 25,000U/m<sup>2</sup>) every 48h x 10 doses without glucocorticoids. Clinical reactions were noted and photographed and ammonia concentrations and asparaginase activities measured.

**Results:** All pigs developed hypersensitive reactions (skin flushing, rash) and ammonia toxicity

(neurologic changes, vomiting) that became more severe with the later doses. Asparaginase caused a rapid increase in serum ammonia, worsening with cumulative doses (Figure). Pre-asparaginase serum ammonia increased from  $29\mu\text{M}\pm 11$  prior to any asparaginase to  $144\mu\text{M}\pm 62$  prior to the 10<sup>th</sup> dose.

**Conclusion:** Intravenous asparaginase causes hypersensitivity and hyperammonemia in the pig model which mimic infusion reactions in humans. Intravenous asparaginase should be administered slowly to reduce the spike in ammonia, and ammonia clearance monitored.



Poster # 699

## Measuring Levels of Stress in Parents of Children with Cancer who have Recently Completed Treatment

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**Background:** The transition to off-therapy follow-up, is a stressful event for parents and/or primary caregivers (henceforth referred to as parents) of children with cancer. The psychosocial needs of parents after therapy have received limited attention in the United States with only 3 published quantitative studies, the largest with 35 parents. We recruited a transition care coordinator (TCC) to quantitatively screen parents at end of therapy, and to develop interventions.

**Objectives:** To assess parent stress at various timepoints after thier child's cancer treatment is completed, as well as to see if it increeases or decreases over the course of these timepoints.

**Design/Method:** After informed consent, a standardized questionnaire, the Psychosocial Assessment Tool (PAT) was administered to parents at therapy completion (T1), 6 months later (T2) and 1 year later (T3). The TCC provided universal intervention to all families with an end of therapy binder containing a treatment summary, follow-up roadmaps, information on late effects, and survivor scholarships. Based on their PATscores, some parents were provided

intervention specific to symptoms (targeted intervention) or referred to a behavioral health specialist (clinical intervention).

**Results:** PAT was administered to 34 parents; at T1 women (n=24) scored 67% higher than men (n=10). Parents experienced worry and anxiety (71%) and sadness/depression (68%). In addition, parents reported post-traumatic stress symptoms of, re-experiencing (27 %) and hyper-vigilance (44 %), and 35 % were found to warrant targeted or clinical intervention, facilitated by the TCC. 50% of parents (n=12) have scored higher on the T2 than the T1. 3 Parents were removed from study due to their child's disease recurrence.

**Conclusion:** This study was initiated in October 2013 using a TCC and PAT screening tool. Results suggest greater stress on mothers after therapy, with a substantial proportion of parents having symptoms of PTSS after therapy.

Poster # 700

## **EPIGENETIC REGULATION OF CELLULAR PROLIFERATION IN ACUTE LYMPHOBLASTIC LEUKEMIA BY IKAROS AND HDAC1**

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**Background:** *IKZF1* (Ikaros) is a major tumor suppressor and reduced Ikaros function is a poor prognostic marker in pediatric B-cell acute lymphoblastic leukemia (B-ALL). Ikaros regulates gene expression *via* chromatin remodeling. In *vivo*, Ikaros forms a complex with histone deacetylases (HDAC1 and/or HDAC2) and the NuRD complex. The mechanisms by which Ikaros exerts tumor suppressor function and regulates gene expression are unknown.

**Objectives:** To identify Ikaros target genes and determine Ikaros role in chromatin remodeling in B-ALL.

**Design/Method:** We performed: chromatin immunoprecipitation with next-generation sequencing (ChIP-SEQ) and ChIP assay with qPCR (qChIP) to identify and confirm the binding profile of Ikaros and HDAC1 in Nalm-6 (B-ALL cell-line) and in primary B-ALL cells; qPCR to identify gene expression of Ikaros targets in Nalm-6 transduced with Ikaros retrovirus, and Ikaros knockdown cells by shRNA; luciferase reporter assay using promoters of target genes; serial qChIP assays spanning Ikaros target gene promoters.

**Results:** Ikaros binds promoter regions of genes critical for cell-cycle progression (CDC2, CDC7, CDC20, CDK2, CDK6, and CCNE2) in B-ALL primary cells. Luciferase reporter assays demonstrated transcriptional repression of these genes by Ikaros. Overexpressing Ikaros resulted in transcriptional repression while downregulating Ikaros increased the expression of Ikaros target genes that control cell-cycle progression. Global epigenetic mapping demonstrated Ikaros binding at the promoter regions of cell-cycle genes resulting in formation of either H3K27me3 or H3K9me3 -repressive epigenetic markers, with loss of H3K9 acetylation and transcriptional repression. qChIP analysis of target gene promoters revealed that H3K27me3 is associated with Ikaros and HDAC1, but H3K9me3 with Ikaros binding alone. Over 80% of H3K27me3 modifications at promoter regions demonstrate HDAC1 binding at surrounding sites. Trichostatin (HDAC inhibitor) treatment of Nalm-6 reduced levels of H3K27me3 (Western blot).

**Conclusion:** We identified two distinct mechanisms for regulation of chromatin remodeling and gene expression by Ikaros in leukemia. Ikaros binds to promoters of target genes and forms



repressive chromatin by: direct binding (increased H3K9me<sup>3</sup>) or recruitment of HDAC1 (increased H3K27me<sup>3</sup>). Ikaros down-regulates cell-cycle progression by repressing transcription of cell-cycle promoting genes. This novel Ikaros-mediated epigenetic regulation of gene expression contributes to tumor suppression in B-ALL.

Poster # 701

## **RISK FACTORS FOR CENTRAL LINE ASSOCIATED BLOOD STREAM INFECTIONS IN CHILDREN WITH ACUTE MYELOGENOUS LEUKEMIA: A SINGLE INSTITUTION REPORT**

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**Background:** Central-line-associated bloodstream infections (CLABSI's) are a challenge in Acute Myelogenous Leukemia (AML) in which over 50% of patients develop bacteremia during their treatment, and previous clinical trials show high infection-related mortality.

**Objectives:** To understand the epidemiology and risk factors associated with the development of CLABSI in children with AML.

**Design/Method:** We retrospectively reviewed all patients with AML over a five year period between 2007 and 2011 at Children's Hospital Colorado. Cases and controls were classified based on the presence of a CLABSI as defined by the National Healthcare Safety Network.

**Results:** Of 48 patients in the study, 65% developed at least one CLABSI during therapy. The majority of CLABSI were due to oral or gastrointestinal organisms (78.2%). *Streptococcus viridians* accounted for 38.2% of infections and gastrointestinal organisms, primarily *E. coli*, *Klebsiella*, and *Enterobacter*, accounted for 38.2%. Skin organisms accounted for 14.5%, all coagulase negative staphylococcus. Fever ( $p<0.0001$ ), severe neutropenia ( $p=0.024$ ), HSV lesions ( $p=0.034$ ), diarrhea ( $p=0.029$ ), number of line entries ( $p=0.003$ ) and receipt of blood products ( $p=0.006$ ) in the previous 4-7 days, not receiving antibiotics ( $p=0.02$ ), and Intensification cycles of chemotherapy ( $p=0.002$ ) all correlated with CLABSI. In contrast, central line type, mucositis, typhlitis, nutritional status, TPN use, foreign body presence, and remission status did not correlate with development of CLABSI. On multivariate analysis, the strongest risk factors associated with CLABSI were diarrhea (OR 6.3, 95%CI 1.6 – 25.2), receipt of blood products in preceding 4-7 days (OR 8.2, 95%CI 2.8 –23.8), not receiving antibiotics (OR 7.2, 95%CI 2.5 –20.9), and chemotherapy cycle (OR 3.5, 95%CI 1.5 –8.6). CLABSI led to increased morbidity with 14 cases (29.2%) and 2 controls (1.9%) requiring transfer to the pediatric intensive care unit ( $p<0.0001$ ). There was a trend toward increased mortality with CLABSI.

**Conclusion:** Intensified line care efforts cannot eliminate CLABSIs in AML patients. Exploring the role of mucosal barrier breakdown in the development of CLABSIs may provide novel prevention strategies. Current antibiotic treatment was protective against CLABSI ( $p=0.022$ ) suggesting that antibiotic prophylaxis may be one effective strategy for prevention of CLABSIs, supporting ongoing trials in this patient population.

Poster # 702

Jennifer Snaman, Doralina Anghelescu, Luis Trujillo, April Coan, Ying Yuan, Justin Baker



**Background:** Patient controlled anesthesia (PCA) is increasingly used to help manage pain in pediatric cancer patients. The role of PCA is especially important in the treatment of patients with refractory or progressive disease and escalating pain at the end of life. However, the description of the use of opioid PCA for management of pain in this population been limited.

**Objectives:** The study objectives were: 1) to describe the patients treated with opioid PCA during the last two weeks of life; 2) to define and compare the morphine-equivalent doses (MED) (mg/kg/day) used at two weeks prior, one week prior to death, and the day of death in this group; and 3) to describe and compare the pain scores within this group of patients at various time points prior to death.

**Design/Method:** The study was a retrospective chart review of all inpatients at our pediatric oncology institution that used PCA during their last 2 weeks of life between February 2004 and January 2011.

**Results:** The mean MED (mg/kg/day) (SD) at two weeks prior to death and the day of death were 10.7 (17.9) and 19.0 (25.8). The mean MED increased during the last two weeks of life and last week of life (mean change [SD] of 6.3 [15.9],  $p=0.07$  and mean change [SD] of 3.3 [19.6],  $p=0.014$ , respectively). There was a significant change in the mean pain scores over the last two weeks of life with the highest pain score on the day prior to the last day of life.

**Conclusion:** Children and young adults with cancer experience a high opioid requirement at the end of life and a significant increase in the use of opioids during the final days of life. The mean MED found in this study is much higher than previously reported. Despite efforts to control pain with titration of opioid PCA, pain scores are high and often rise. Opioid rotation and addition of adjuvant medication should be considered in patients with rapidly escalating opioid requirements to control pain and ease suffering at the end of life.

Poster # 704

## **HISTONE PROFILING IN LEUKEMIA USING LC-MS/MS**

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**Background:** Epigenetic changes, including aberrant histone modifications, play a crucial role in malignant transformation and contribute to relapsed and refractory disease. There are over 160 known histone modifications, most of which remain poorly explored. The protein network that regulates these PTMs offers potential therapeutic opportunities through pharmacologic inhibition. Most mutations in the histone modifying enzymes have been identified by whole genome sequencing efforts. More recently, mass spectrometry has been proposed as a technique to identify patterns of histone modifications in cancer – Jaffe et al (Nature Genetics, 2013) developed a mass spectrometry approach to global chromatin profiling using leukemia cell lines.

**Objectives:** We adapted the methods established by Jaffe et al to histone modification profiling using small amounts of primary material from leukemia patients. Our initial analysis focused on 5 sets of paired patient samples from diagnosis and relapse.

**Design/Method:** Our work flow involves enrichment of blasts by ficoll centrifugation or fluorescent activated cell sorting, isolation of highly purified histones from patient samples using a resin column followed by gel electrophoresis, digestion with ArgC and Trypsin, and nanoscale

liquid chromatography followed by tandem mass spectrometry (nano LC-MS/MS). We established a leukemia cell line panel labeled with heavy (13C6) arginine and heavy (13C6) lysine for SILAC based internal standardization.

**Results:** Initially focusing our analysis on H3K27 and H3K36 methylation and using published retention times and m/z ratios, we were able to identify almost all major combinatorial acetylation and methylation states for the two residues. We also identified methylation/acetylation states for H3K9 (ac), H3K14 (ac), H3K18 (ac, me), H3K23 (ac), and H3K79(me, me2). Preliminary experiments using a cell line with an activating NSD2 mutation correctly identified increased H3K36 dimethylation and decreased H2K27 trimethylation compared to the SILAC standard.

**Conclusion:** We have shown that mass spec can be used to create histone profiles in patient samples, even with very limited starting material. Preliminary analysis has shown that comparing histone profiles in a single patient from diagnosis and relapse could provide information on epigenetic mechanisms of relapse.

Poster # 705

## **ROLE OF ASPARAGINASE SERUM MONITORING IN MANAGING HYPERSENSITIVITY REACTIONS TO PEGASPARGASE IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA**

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**Background:** Hypersensitivity reactions pose a significant challenge in optimizing asparaginase therapy. Serum monitoring of asparaginase concentrations may serve as an objective tool to mitigate risk in planning an asparaginase rechallenge despite prior history of hypersensitivity symptoms.

**Objective:** To describe use of asparaginase serum levels to aid in the management of pediatric acute lymphoblastic leukemia (ALL) patients with hypersensitivity reactions to pegaspargase.

**Design/Method:** Patients with a diagnosis of precursor-B ALL treated at Children's of Alabama from 2012 to 2014 were eligible. Detailed chart review was conducted on 4 patients who underwent asparaginase serum level monitoring following a hypersensitivity reaction to pegaspargase.

**Results:** Four children developed hypersensitivity reactions with intravenous pegaspargase during consolidation therapy. Symptoms included cough, rash, vomiting and agitation. No premedication was given before these doses. Three patients were able to resume and complete their infusions on the same day following use of histamine blockers and steroid medications. Serum asparaginase levels obtained within 4 to 7 days post-dose were deemed therapeutic (0.65-1.59IU/ml). These 3 patients were then successfully rechallenged on subsequent pegaspargase administrations using premedication and slower infusion rates, and asparaginase levels remained in therapeutic range. A fourth patient successfully completed pegaspargase infusion with premedication on the day following his initial reaction. However, asparaginase concentration was undetectable on Day 7 post-dose, and he was switched to Erwinia asparaginase (ErwA) without further events.

**Conclusion:** Hypersensitivity reactions have historically prohibited optimization of asparaginase as symptoms are often believed to be due to antibodies that would also result in clearance of the medication and suboptimal drug exposure (neutralizing antibodies). As evidenced by our small

cohort of patients, we have seen that in those patients whose symptoms are controlled with premedications and slower infusion rates, 3 out of 4 patients were found to have therapeutic asparaginase levels despite the hypersensitivity symptoms. The commercially available asparaginase assay can serve as an objective, cost-effective tool to proactively monitor the efficacy of asparaginase therapy while treating mild to moderate hypersensitivity symptoms.

Poster # 706

## **INTERFERENCE WITH BONE MARROW STROMA DERIVED CXCL12 INCREASES APOPTOSIS IN ALL CELLS AND ENHANCES ANTILEUKEMIA EFFECTS OF CHEMOTHERAPY DRUGS**

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**Background:** Bone marrow stroma provides a favorable microenvironmental niche for ALL cell survival. We and others have demonstrated that bone marrow stromal cells contribute to prevention of apoptosis in ALL cells.

**Objectives:** Identify potentially “drug-able” molecules derived from marrow stromal cells that contribute to prevention of ALL cell apoptosis.

**Design/Method:** We have developed an in vitro system to identify stromal gene products that deliver antiapoptotic signals to ALL cells. Primary human ALL cells are co-cultured with human stromal cells. We manipulate stromal cells with siRNA directed against candidate stromal cell genes, and then measure viability and apoptosis in ALL cells by flow cytometry.

**Results:** (1) Knockdown of stroma cell CXCL12 with siRNA increases ALL cell death in the coculture system. As measured by quantitative reverse transcriptase PCR stromal cell CXCL12 mRNA was reduced approximately 75% by siRNA treatment. In 7 of 12 experiments we observed reduction of ALL cell survival on treated stroma. The magnitude of effect was 32-39% increase in ALL cell death. (2) Pharmacologic interference with stromal cell CXCL12/ALL cell CXCR4 interactions increases ALL cell death. The gene knockdown experiments suggested a potential role for CXCL12 in prevention of ALL cell apoptosis. To further test this we tested the effect of plerixafor, a specific inhibitor of CXCL12/CXCR4 interactions, on survival of ALL. In a dose dependent manner (25 - 100 micromolar) we observed a 31-39% reduction in ALL survival in stromal cocultures including plerixafor. (3) Blockade of CXCL12/CXCR4 interactions with plerixafor increases the antileukemia effect of some chemotherapy drugs. In our stromal cell/ALL coculture system we have identified the effective in vitro concentrations of dexamethasone, methotrexate, vincristine, l-asparaginase and 6-mercaptopurine. We measured the impact of combination of plerixafor (LD10) and these individual drugs (used at approximately the LD50 concentrations). We observed increased antileukemia effects related to plerixafor for dexamethasone (51% increase), methotrexate (56% increase), vincristine (45% increase), and 6-mercaptopurine (59%). We did not observe increased effectiveness with l-asparaginase.

**Conclusion:** Marrow stromal cell-produced CXCL12 may contribute to prevention of apoptosis in human ALL cells. Pharmacological interference with its effect may enhance the effectiveness of some conventional chemotherapy drugs.

Poster # 707

## INTRAVENOUS IMMUNOGLOBULIN ADMINISTRATION DURING MAINTENANCE CHEMOTHERAPY IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA: EVALUATION OF INDICATION, USE, AND EFFICACY

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**Background:** There can be a marked immunosuppression during the maintenance phase of chemotherapy in pediatric Acute Lymphoblastic Leukemia (ALL). Intravenous immunoglobulin (IVIG) has been used to try to reduce infectious complications, but currently there is no consensus regarding its use.

**Objectives:** Describe the use of IVIG during maintenance chemotherapy in pediatric ALL, compare the IVIG versus non-IVIG groups

**Design/Method:** A multicenter, retrospective cohort of consecutive patients treated from 2008 to 2014. Data were collected via review of our electronic medical record and treatment roadmaps. Comparisons using nonparametric statistics, including Chi-Square, Wilcoxon rank sum and signed ranked tests were used to compare differences between the IVIG group and the non-IVIG group.

**Results:** One hundred eighteen patients were included in the study, 53% male, age range nine months to 19 years. Thirty-six patients (30%) received IVIG during maintenance chemotherapy for the following indications: decreased immunoglobulin levels in a clinically stable patient (n=11), infection prior to start of maintenance chemotherapy (n=5), infection during maintenance chemotherapy (n=16), and prophylaxis for viral exposure (n=4). There was a significant increase in immunoglobulin levels from the time of initiation to discontinuation of IVIG (mean 404 vs 672;  $P = 0.008$ ). Patients received an average of 10.5 (range 1-29) doses of IVIG. Between the five medical centers, there was a significant difference in percent of patients that received IVIG ( $P = 0.002$ ), the average number of doses given ( $P = 0.008$ ), and the number of times immunoglobulin levels were checked ( $P < 0.001$ ). Prior to maintenance chemotherapy, the IVIG group had more episodes of bacteremia (mean 0.89 vs 0.26;  $P < 0.001$ ) and total days of hospitalization for infection (mean 20.4 vs 11.5;  $P = 0.011$ ). During maintenance chemotherapy, the IVIG group had more days of hospitalization (mean 11.1 vs 6.7;  $P = 0.032$ ), but no differences in episodes of bacteremia (mean 0.25 vs 0.15;  $P = 0.247$ ), or outpatient antibiotic use (mean 4.8 vs 4.7;  $P = 0.892$ ).

**Conclusion:** The use of IVIG varies significantly between providers. The IVIG group had more infectious complications prior to maintenance chemotherapy; during maintenance chemotherapy, their course was more comparable to the non-IVIG group.

Poster # 708

## ROLE OF WNT/ $\beta$ -CATENIN PATHWAY IN THERAPY RELATED MYELOID NEOPLASMS (T-MDS/AML) WITH 5Q DELETION: A NEW THERAPEUTIC TARGET

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**Background:** The 5 year survival for t- AML is 25%. Del(5q) is a common recurring cytogenetic abnormality noted in up to 25% of pediatric patients with t-MDS/t-AML. The underlying mechanisms leading to leukemia are incompletely understood. Del 5q is associated with haplo-insufficiency of APC gene in hematopoietic stem cells (HSCs) which leads to MDS-like disease in mice (Blood 2010). APC regulates the function of HSCs through  $\beta$ -catenin dependent mechanisms (Blood 2013).

**Objectives:** To show effects of blocking the Wnt pathway by inhibition of  $\beta$ -catenin on del 5q myeloid leukemia.

**Design/Method:** UoCM1 and MDS-L are human AML cell lines with del 5q and REH and MV4-11 without 5q deletion. Western Blot shows that the expression of  $\beta$ -catenin is higher in UoCM1 and MDS-L versus REH and MV4-11. We treated all four cell lines with 100 $\mu$ M Indomethacin which was shown to inhibit  $\beta$ -catenin. Lentiviral particles expressing a control empty backbone or  $\beta$ -catenin targeting inhibitory shRNA were transduced into UoCM1, MDS-L and REH and complete  $\beta$ -catenin knockdown achieved. The cells were analyzed for effects on growth, cell cycle and apoptosis.

**Results:** The UoCM1 and MDS-L cells showed significantly more growth inhibition in culture, decreased colony formation, increased apoptosis and decreased cell proliferation determined by cell-cycle compared to REH and MV4-11 after treatment with 100 $\mu$ M Indomethacin. UoCM1 and MDS-L cells transduced with  $\beta$ -catenin shRNA showed significantly more growth inhibition in culture, decrease in the fractions of cells in S and G2/M phase, increase in apoptosis and decrease in colony formation compared to control vector transduced cells. In contrast, REH demonstrated comparable growth, no difference in distribution in cell cycle and a similar frequency of apoptosis and colony formation in  $\beta$ -catenin inhibited and control cells.

**Conclusion:** 5q del in human AML cell line leads to up-regulation of  $\beta$ -catenin. Blockade of Wnt pathway by pharmacologic and shRNA mediated inhibition of  $\beta$ -catenin suppresses cell growth and leukemia regenerating ability and induces apoptosis in a human AML cell lines with 5q del. This discovery paves the way for new therapeutic strategies targeting  $\beta$ -catenin and improving survival in 5q del t-AML/MDS.

Poster # 709

## **L-ASPARAGINASE INDIVIDUALIZED DOSING AND SWITCHING IN ALL: AN NNT ANALYSIS**

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**Background:** L-asparaginase is an important component of chemotherapy for pediatric acute lymphoblastic leukemia (ALL). Vrooman et al<sup>1</sup> randomized newly-diagnosed ALL patients to fixed-dose (FD) (25,000 IU/m<sup>2</sup>) *Escherichia coli* L-asparaginase (EC-Asnase) versus individualized dosing (ID) with an initial dose of 12,500 IU/m<sup>2</sup>, and subsequent dose adjustments to maintain nadir serum asparaginase activity (NSAA) between 0.1 and 0.14 IU/mL. ID patients had a statistically significantly improved 5-year event-free survival (EFS;  $P=.04$ ).

**Objectives:** To calculate the number needed to treat (NNT) to prevent one event (relapse or death) for ID of EC-Asnase compared with an FD approach and to calculate the NNT for switching asparaginase preparations in response to an NSAA threshold suggestive of subclinical hypersensitivity (silent inactivation).

**Design/Method:** NNT was calculated as the reciprocal of the absolute risk reduction (1/ARR),

where ARR is equal to the control (FD) minus experimental (ID) event rates.<sup>1,2</sup> We also calculated the NNT for switching asparaginase preparations in ID patients with NSAA <0.1 IU/mL on successive determinations despite dose adjustment or when coupled with EC-Asnase antibody positivity. NNTs were compared with those for other pediatric oncology interventions. For comparison, a literature search was conducted to identify randomized controlled trials (RCTs) using interventions in other pediatric hematologic and solid malignancies reported in the past 10 years.

**Results:** In Vrooman et al,<sup>1</sup> the 5-year EFS for the FD and ID groups was 82% and 90%, respectively (NNT=13 for ID compared with FD). In addition, FD patients with levels <0.1 IU/mL who never switched preparations had a 5-year EFS of 76% versus 95% for ID patients who switched preparations for silent inactivation (NNT=5). Five RCTs in ALL and other pediatric cancers (acute myeloid leukemia or myelodysplastic syndrome associated with Down syndrome; nonmetastatic rhabdomyosarcoma; nonmetastatic osteosarcoma; high-grade astrocytomas) had outcome measures with NNTs ranging from 4 to 50.

**Conclusion:** These NNT values for ID and for switching asparaginase preparations based on NSAA levels suggestive of silent inactivation resemble those for well-accepted oncology treatments, highlighting the potential value of monitoring to prospectively identify suboptimal asparaginase activity.

**References:** 1. Vrooman et al. *JCO* 2013; 31(9):1202-1210. 2. Laupacis et al. *NEJM* 1988; 318(26):1728-1733.

Supported by Jazz Pharmaceuticals

Poster # 710

## GENERATING POTENT CHIMERIC ANTIGEN RECEPTOR T CELLS USING INTERLEUKIN 15 RECEPTOR ALPHA SIGNAL AS A CO-STIMULATORY DOMAIN

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**Background:** Chimeric antigen receptor (CAR) T cell therapy is an innovative form of adoptive immunotherapy which involves constructing chimeric T cell receptors with antigen binding domain derived from scFv portion of antibody and activation domain derived from CD3 $\zeta$  of T cell receptor with or without other co-stimulatory domains using advanced genetic engineering techniques. Efforts to improve these cells involve modulating the co-stimulatory domains, incorporating cytokine growth factors or using immunotherapy checkpoint blockers.

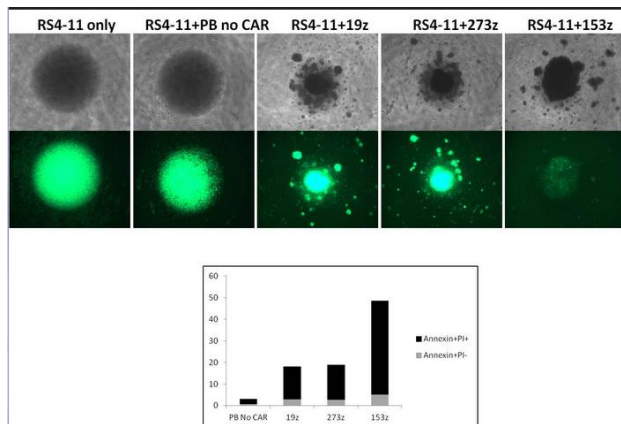
**Objective:** To generate CART cells with increased potency using cytokine receptor signals as co-stimulatory domains.

**Design/Method:** We designed novel third generation chimeric antigen receptor T cell targeting CD19 using interleukin 15 receptor alpha (IL15R $\alpha$ ) signal as a co-stimulatory domain in addition to CD28 and CD3 $\zeta$  with lentiviral vectors. We used GFP labeled CD19 positive target cell line (RS4-11) and calcein AM labeled leukemia cells and tested killing efficacy with FACS based killing assay.

**Results:** CART cells with IL15R $\alpha$  signal effectively killed target cells as evidenced by annexin V/PI apoptosis and disappearance of GFP labeled target cells when compared to those with 4-1BB and CD27 co-stimulatory signals. These cells retained their killing efficacy when more target cells were added. We found that these cells proliferated to a higher extent using CFSE

labeled assay and displayed enhanced effector activities illustrated by intracellular cytokine staining for IL-2 and IFN $\gamma$ .

**Conclusion:** CART cells with IL15R $\alpha$  signal have increased killing efficacy. **Future Direction:** To study exhaustion mechanisms to make these cells last longer and generate these CART cells for solid tumors.



Poster # 711

## PEDIATRIC-INSPIRED VERSUS HYPERCVAD PROTOCOLS FOR PHILADELPHIA-NEGATIVE ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) IN ADOLESCENTS AND YOUNG ADULTS (AYA): AN NNT ANALYSIS

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**Background:** ALL treatment outcomes in adolescents and young adults (AYAs, ages 16-39 years) are poorer relative to children due to age-associated cytogenetic features and treatment differences. While treatment of AYA ALL patients with hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (hyperCVAD) is common in the US, noncomparative data suggest that outcomes may be inferior to pediatric-inspired treatment protocols containing higher doses of nonmyelosuppressive agents including L-asparaginase.

**Objectives:** Calculate the number-needed-to-treat (NNT) to prevent one relapse or death for pediatric-inspired treatment vs hyperCVAD in AYA ALL patients.

**Design/Method:** No comparative clinical trials were found in a comprehensive review of published literature to identify outcomes for Philadelphia-negative AYA ALL patients undergoing pediatric-inspired vs hyperCVAD first-line treatments. Progression-free survival (PFS) was estimated for these regimens based on two studies with similar patient populations<sup>1,2</sup> by fitting Weibull curves to the published PFS rates. The NNT to prevent one relapse or death was calculated using these estimates. NNT is the inverse of the absolute risk reduction (ARR) associated with an experimental intervention relative to controls ( $1/ARR$ ), where ARR is equal to control event rate (CER) minus experimental event rate (EER) ie,  $ARR = CER - EER$ <sup>3</sup>.

**Results:** Five-year PFS estimated from the relative survival curves (Figure) were 0.44 for hyperCVAD and 0.62 for pediatric-inspired regimens, translating into an NNT of 6.

**Conclusion:** An indirect comparison of published literature in similar populations suggests that pediatric-inspired regimens may have better clinical outcomes than hyperCVAD, with a low

NNT estimate of 6.

**References:** 1) Kantarjian, *Cancer*, 2004; 2) Storrington, *BJH*, 2009; 3) Laupacis, *NEJM*, 1988.

Supported by Jazz Pharmaceuticals

Poster # 712

## **MLL-REARRANGED ALL CELLS RELEASE HIGH MOBILITY GROUP BOX 1, AN IMMUNOLOGIC DANGER SIGNAL THAT SUPPRESSES MACROPHAGE FUNCTION AND CAR T CELL KILLING IN AN IN VITRO MODEL OF THE TUMOR MICROENVIRONMENT**

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**Background:** The prognosis of infant MLL-rearranged ALL remains poor despite attempts at chemotherapy intensification. The short time-to-relapse suggests an immune-privileged tumor microenvironment (TME) that may limit the effectiveness of currently available, T-cell based immunotherapy. Identification of potential targets to overcome immunosuppression would increase the effectiveness of T-cell based therapy and improve cure rates in MLL-ALL.

**Objectives:** This study tests the hypothesis that release of the immunologic danger signal high mobility group box-1 (HMGB1) by MLL-ALL inhibits macrophages inflammatory signaling and suppresses anti-leukemia T-cell function.

**Design/Method:** Primary ALL cells were isolated from the diagnostic marrow specimens of 5 MLL-ALL and 5 standard-risk ALL (SR-ALL) pediatric patients. Release of HMGB1 before and after 2uM doxorubicin treatment was measured by ELISA in MLL-ALL, SR-ALL and healthy donor B cells and compared by Student t-test. Inflammatory cytokine production and NFkB protein expression in bone marrow macrophages (BM-MF) added to ALL cultures was measured by ELISA and Western blot. Proliferation, degranulation and target cell killing of CD19 chimeric antigen receptor T-cells (CAR T, courtesy Dr. Stephen Forman) treated with conditioned media from these co-cultures was measured by flow cytometry and compared by Kruskal-Wallis test.

**Results:** MLL-ALL produced significantly more HMGB1 than SR-ALL both before ( $18.9 \pm 0.4$  vs  $1.2 \pm 0.1$  ng/ml,  $p < 0.05$ ) and after ( $56.6 \pm 2.7$  ng/ml vs.  $10.7 \pm 0.3$  ng/ml,  $p < 0.0001$ ) doxorubicin treatment. BM-MF co-cultured with HMGB1-producing MLL-ALL cells expressed less total and phosphorylated NFkB protein by Western blot, and their production of the canonical inflammatory cytokine IL1beta was decreased to undetectable levels ( $p < 0.0001$ ). CAR T cells treated with conditioned media from co-cultures of HMGB1-producing MLL-ALL showed an increase in PD-1 expression (5-fold increase in mean fluorescence intensity,  $p < 0.05$ ), decrease in proliferation ( $45.2 \pm 5.9\%$  decrease,  $p < 0.05$ ) and reduction in target cell killing compared to those treated with conditioned media from SR-ALL cells.

**Conclusion:** HMGB1 produced preferentially by MLL-ALL cells, results in impairment of macrophage inflammatory signaling and reduced cytolytic function of CAR T cells in an *in vitro* model of the ALL microenvironment. Studies are underway to determine whether therapeutic neutralization of HMGB1 can improve immune responses to MLL-ALL.

Poster # 713

## **BORTEZOMIB, DEXAMETHASONE, MITOXANTRONE AND VINOBLASTINE**



# (BDMV): A HIGHLY ACTIVE REINDUCTION REGIMEN FOR CHILDREN WITH RELAPSED ALL AND ASPARAGINASE INTOLERANCE

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**Background:** Children with relapsed ALL traditionally receive Vincristine-Prednisone-L-Asparaginase-Doxorubicin (VPLD) reinduction chemotherapy similar to contemporary induction regimens. However, 4-18% of patients are unable to receive VPLD secondary to asparaginase intolerance. We report our institution's experience with a promising reinduction regimen BDMV for children with relapsed ALL who are unable to receive VPLD.

**Objectives:** This is a single institution, retrospective study of the safety and activity of BDMV in patients with relapsed ALL. Complete remission (CR) and adverse events (AEs) following reinduction were study endpoints.

**Design/Method:** Patients treated with BDMV between 2012 to 2014 were identified following IRB approval. Response and toxicities were assessed by review of medical records. Standard response criteria were employed and AEs were graded based on NCI CTCAEv4.0.

**Results:** Eleven patients were included in this study. 10 patients had M3 marrow disease. One patient was in MRD+ CR prior to BDMV and not included in response analysis. Patient characteristics and outcome are detailed in Table 1. Patients had an average of 1.6 treatment attempts (Range: 1-4) prior to BDMV. 7/10 patients achieved CR after one cycle of BDMV, with 4/7 achieving MRD negativity. Grade  $\geq 3$  infections were seen in 82% of patients, including one grade 5 sepsis. Other common Grade  $\geq 3$  toxicities included GI 36% and metabolic 18%.

**Conclusion:** BDMV is a highly active reinduction regimen for children with relapsed ALL who cannot receive VPLD. Toxicity profile is as expected for this patient population. Further prospective clinical trials are warranted to evaluate the safety and efficacy of BDMV.

Table 1. Patient Characteristics and Response

	All Patients	1 <sup>st</sup> Relapse		2 <sup>nd</sup> Relapse
		Early Relapse*	Late Relapse*	
N	11	7	2	2
Mean age (year) at time of therapy	14.6 (range 0.75 - 23)	12.3 (range 1 - 19)	18.2 (range 12 - 23)	19.8 (range 19-20)
Gender				
Male	6 (55%)	3 (43%)	1 (50%)	2 (100%)
Female	5 (45%)	4 (57%)	1 (50%)	0
Site of Relapse				
Isolated BM	7 (64%)	4 (57%)	2 (100%)	1 (50%)
Combined	4 (36%)	3 (43%)	0	1 (50%)
Mean CR1 duration (months)	N/A	14.2 (range 3-34)	46.5 (range 42-51)	N/A
Mean Duration of CR2 (months)	N/A	N/A	N/A	33 (range 22-44)
Response Data (N=10) <sup>^</sup>				
CR	7 (70%)	3 (50%)	2 (100%)	2 (100%)
MRD negative <sup>*</sup>	4 (57%)	1 (33%)	1 (50%)	2 (100%)
MRD positive	3 (43%)	2 (67%)	1 (50%)	0
Refractory Disease	2 (20%)	2 (33%)	0	0
Induction Death	1 (10%)	1 (17%)	0	0
Marrow Recovery				
ANC recovery (days)	34.5 (range 26-50)	38.2 (range 26-50)	29 (range 27-31)	31 (range 27-35)
Platelet recovery (days)	32.1 (range 22-40)	36.7 (range 34-43)	24 (range 23-25)	31 (range 22-40)

<sup>\*</sup> Early relapse was defined as bone marrow or combined relapse within 36 months of diagnosis;

<sup>\*</sup> late relapse was defined as bone marrow or combined relapsed > 36 months of diagnosis

<sup>\*</sup> Negative MRD was defined as < 0.01% abnormal cells within mononucleated cells by

multicolor flow cytometry from our institution.

<sup>^</sup> One patient was in MRD positive CR prior to BDMV and was not included in the response analysis. The patient remained to be MRD positive after BDMV.

## MODIFYING ENGAGER T-CELLS TO EXPRESS COSTIMULATORY MOLECULES FOR THE IMMUNOTHERAPY OF CD19 POSITIVE MALIGNANCIES

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**Background:** Clinical studies with anti-CD19/anti-CD3 bispecific engager proteins, which activate T cells in an antigen-dependent manner, have produced encouraging results for CD19+ malignancies. However, these molecules have short half-lives and enhancing their antitumor effects by co-delivery of costimulatory molecules is impossible. To overcome these limitations we have genetically modified T-cells to secrete bispecific engager molecules (ENG T-cells). ENG T cells are activated in an antigen dependent manner, recruit resident T-cells to tumors, and have antitumor activity in preclinical models.

**Objectives:** The goal of this project was to determine if providing costimulation would enhance the antitumor activity of CD19-ENG T-cells.

**Design/Method:** CD19-ENG T cells were generated by transducing T-cells with a retroviral vector encoding a CD19-specific T-cell engager, and CD19-ENG/Costim T-cells were generated by double transducing T-cells with the previous construct and a 2<sup>nd</sup> retroviral vector encoding the costimulatory molecules 41BBL and CD80. The effector function of the generated T-cells was evaluated *in vitro* and in a xenograft model.

**Results:** CD19-ENG and CD19-ENG/Costim T-cells recognized CD19+ lymphoma (Daudi, Raji) and acute leukemia (BV173) cells as judged by IFN- $\gamma$  secretion. Both ENG T-cell populations produced IL2 in the presence of CD19+ targets expressing CD80 and CD86 (Daudi and Raji). However, CD19-ENG/Costim T-cells produced higher levels of IL2 in comparison to CD19-ENG T-cells after stimulation with BV173 (CD19+CD80-CD86-). ENG and ENG/Costim T-cells specific for an irrelevant antigen (EphA2) did not produce cytokines, confirming antigen dependence. Specificity was confirmed in cytotoxicity assays. *In vivo* anti-tumor activity of CD19-ENG T-cells and CD19-ENG/Costim T-cells was assessed in a BV173/NSG xenograft model. Therapy with CD19-ENG T-cells on day 7 post tumor injection resulted in the cure of all mice. Only 1/10 mice was alive on day 80 when T-cells were injected on day 14. Treatment with CD19-ENG/Costim T-cells starting on day 14 resulted in long-term survival of 7/10 mice. Control T-cells had no antitumor effects.

**Conclusion:** CD19-ENG T cells and CD19-ENG/Costim T-cells have potent antitumor activity. Provision of costimulation further enhanced antitumor effects. Genetically modifying T-cells to express engagers and additional molecules to enhance their effector function may present a promising alternative to current CD19-targeted immunotherapies.

Poster # 715

## EXCELLENT OVERALL SURVIVAL (100%) IN TURKISH CHILDREN WITH STANDART RISK ACUTE LYMPHOBLASTIC LEUKEMIA

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**Background:** Outcome of childhood acute lymphoblastic leukaemia (ALL) could be improved by intensification of conventional chemotherapy and supportive therapies in recent years. However, there is limited data about the very successful long-term treatment outcome of ALL in newly industrialized countries.

**Objectives:** Our study was designed to assess survival data and demographic features.

**Methods/Design:** We evaluated the results of the standard risk ALL protocols according to the Children's Oncology Group (CCG-1991, COG-0331, COG-0932) used between 1999 and 2014 at Pediatric Hematology/Oncology Departments of Istanbul University Oncology Institute and Bezmialem Vakif University. A retrospective analysis of 52 children newly diagnosed ALL with standard risk was evaluated.

**Results:** The median age was 4 years (1,5-8 yrs) among 52 (31 boys, 23 girls) patients with "standard risk" according to the Schultz criteria. Median follow-up time was 3.5 years (6 mo. -15 yrs). Five patients (9,6%) were slow early responders and had more intensive chemotherapy. One patient who leaved treatment at the beginning of the maintenance had testicular relapse after 5 years. Event free survival (EFS) at 5 years was 94,4% and overall survival (OS) was 100%. During treatment period 6 pulmonary infection, 4 avascular necrosis, 2 hypoglycemia, 1 hyperglycemia and 1 pancreatitis were seen.

**Conclusion:** This study successfully showed that children with standard risk ALL treated with COG protocols have very encouraging results. The most important point is the outpatient chemotherapy administration after remission, no delay during the treatment and also continuous monitoring of the same experienced clinicians. Our study might contribute both the recognition of outpatient applicable COG protocols to the other centers in developing world and also point out the importance of required follow up of these patients.

Poster # 716

## THE FEASIBILITY OF QUANTITATIVE SENSORY TESTING IN PEDIATRIC ONCOLOGY PATIENTS TREATED WITH VINCRISTINE

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**Background:** Neuropathic pain is a dose-limiting side effect of neurotoxic chemotherapy and can be difficult to differentiate from nociceptive pain. Treatment strategies differ so novel neuropathic pain assessment tools are necessary. Furthermore, the neurobiology of chemotherapy-induced neuropathic pain is poorly understood. Quantitative Sensory Testing (QST) can help differentiate neuropathic from nociceptive pain and assess pain neurobiology. To date, no published data exist using QST in children with cancer.

**Objectives:** In pediatric oncology patients and controls: 1) Determine the feasibility of QST and 2) Collect pilot data on QST outcomes.

**Design/Method:** A cross-sectional, pilot study was conducted. All participants were age  $\geq 7$  years and all oncology patients were treated with vincristine. Participants underwent thermal and mechanical QST on the thenar eminence of the non-dominant hand. QST evaluates the somatosensory system, detecting sensory loss (hyposensitivity) or gain (hypersensitivity). Thermal testing was performed with a computer-assisted device that delivers cold and warm stimuli via a thermode attached to the skin (baseline temperature, 32°C; range, 0-50°C). Mechanical testing was performed using vonFrey monofilaments (force range 0.255 mN

to 1078.731 mN). Feasibility outcomes included: consent rate, time to complete testing, completion rate, and willingness to undergo repeat testing. QST outcomes included: cold pain threshold (°C), heat pain threshold (°C), and mechanical pain threshold (mN) as reported by “method of limits” where participants pushed a button (thermal) or spoke (mechanical) when the progressive stimulus was painful.

**Results:** Of those approached, 33/34 patients and 20/20 controls agreed to participate. Mean age was  $12.2 \pm 3.6$  years for controls and  $13.1 \pm 4.4$  years for patients. All participants completed QST in its entirety. QST took  $37 \pm 12$  minutes to complete and 94.3% of participants agreed to undergo QST again. The mean cold pain threshold was  $14.8 \pm 8.9^{\circ}\text{C}$  in patients versus  $11.8 \pm 10.4^{\circ}\text{C}$  in controls. The mean mechanical pain threshold was  $381.2 \pm 421.1$  mN in patients versus  $503.9 \pm 482.7$  mN in controls.

**Conclusion:** QST in pediatric oncology patients is feasible. These pilot data support larger studies to investigate the observed trends toward cold and mechanical hypersensitivity in pediatric oncology patients treated with neurotoxic chemotherapy.

Poster # 717

## **CAN ETOPOSIDE BE AVOIDED IN SOME PATIENTS WITH HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS?**

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**Background:** Due to its diverse clinical presentations, the diagnosis of Hemophagocytic Lymphohistiocytosis (HLH), a syndrome characterized by uncontrolled inflammation caused by hyperactivation/infiltration of macrophages and T-lymphocytes, is often delayed. The diagnostic approach and management strategies employed by treating centers depend on the available medical expertise and laboratory facilities.

**Objectives:** To describe the disease characteristics, clinical features, treatment and outcome of 8 patients with HLH treated at our rural tertiary children's hospital (*not* a supra-regional HLH referral center) in the last 3 years and then propose a diagnosis/management/referral algorithm to be used in different clinical settings.

**Design/Method:** Case series

**Results:** Of our 8 patients, a known familial HLH (FHLH)-predisposing mutation was detected in 3 patients and HLH-predisposing immunological defects in 2 patients. Seven patients presented with clinical HLH and treatment was initiated in 6 (1 patient expired prior to commencing HLH therapy). One patient, diagnosed with FHLH, underwent bone marrow transplantation prior to developing clinical HLH. Of the 7 patients who had clinical HLH 5 were older than 5 years (1 FHLH), and 2 were younger than 5 (1 FHLH). All 7 had an associated infection/pathology (EBV, HHV6, Histoplasma, Rotavirus, Rickettsia, Spider Bite, and Hemolytic Uremic Syndrome). Five patients satisfied HLH-2004 diagnostic criteria before HLH-directed treatment was commenced and 1 patient satisfied diagnostic criteria after treatment was commenced. Time from admission to commencement of treatment was <48 hours (2 patients), 48-96 hours (2 patients) and >96 hours (2 patients). All 7 patients treated for clinical HLH had serum ferritin levels over 1500ng/mL at diagnosis (5 higher than 3000ng/ml). Only 2 patients had bone marrow hemophagocytosis at diagnosis. 5 patients had IL-2R levels over 3000U/mL. Disease control was achieved in 4 patients with Dexamethasone alone. 2 patients

succumbed to HLH. None of the 3 patients diagnosed with secondary HLH have relapsed.

**Conclusions:** In patients diagnosed and treated early in the course of clinical HLH it may be possible to achieve disease control with Dexamethasone alone thus avoiding the use of Etoposide. We are working towards improving our outcomes by educating inpatient services on disease recognition, contacting a supra-regional center early, and streamlining our pretreatment and send-out investigations.

Poster # 718

## **MANAGEMENT OF PEDIATRIC PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA: SINGLE CENTER EXPERIENCE IN VENEZUELA**

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**Background:** It is well known that survival outcomes of childhood cancers in developing nations have failed to keep pace with those of developed nations, mainly owing to socioeconomic reasons.

**Objectives:** From October 2012 to October 2014, 32 pediatric patients with Acute Lymphoblastic Leukemia were diagnosed and treated at Hospital Militar Dr Carlos Arvelo, Caracas, Venezuela. We followed a pediatric based Protocol (Total XVI St Jude) and treated according to the outlined chemotherapy and supportive measures.

**Design/Method:** Clinical, laboratory, bone marrow immunophenotyping, molecular biology and CSF studies were required for diagnosis and risk stratification, and induction therapy was started promptly in all cases. Close management and follow-up were delivered by a multidisciplinary team in an academic, tertiary care hospital.

Chemotherapy was delivered with no delay, as specified in the Protocol, including the use of pegylated asparaginase (2500 U/m<sup>2</sup>/d) as well as antibiotics and other supportive measures and drugs if needed. All patients, except for one, achieved negative minimal residual disease by the end of induction. Special tests were performed at centralized laboratories and clinical care was provided, both inpatient and outpatient, by a dedicated pediatric oncology ward with 3 pediatric hematologist/oncologists, and the support of an experienced nursing staff and pediatric trainees.

**Results:** Complications were anticipated and managed according to protocol's guidance, and clinicians' criteria. All patients recovered from infectious and other complications. Only one 11 year old girl, who had developed multiple complications, experienced an early relapse to date. There was 1 death, infectious related from a patient with T precursor ALL. The other patients are advanced in their treatment, and doing well.

**Conclusion:** Suitable curative treatment of pediatric patients with acute lymphoblastic leukemia, using modern protocols, is feasible and effective in comprehensive centers in the developing world.

Poster # 719

## **CLINICAL & LABORATORY PROFILE OF HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN CHILDREN FROM A TERTIARY-CARE PEDIATRIC CENTRE IN INDIA**

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**Background:** Hemophagocytic lymphohistiocytosis (HLH) is a potentially fatal disorder resulting from uncontrolled activation of macrophages & lymphocytes.

**Objectives:** To describe clinical & laboratory features of children diagnosed with HLH at a tertiary pediatric centre from a developing country.

**Method:** Retrospective analysis of data of patients (January'05-December'14), who fulfilled the 2009-Filipovich diagnostic criteria were included in this study. Data included clinical history, examination, laboratory investigations, bone marrow aspiration (BMA) reports, perforin levels & granule release assay (GRA). NK cell activity & sIL2R $\alpha$  were not performed at our centre.

**Results:** The cohort consisted of 97 children (61 males & 36 female) with an age group of day 21 to 13.4 years (mean-3.06 years). 21.6% were born of a consanguineous union. Fever was the commonest symptom (93.8%). Symptoms involving abdominal, respiratory, renal & central nervous system (CNS) were seen in 37 (38.1%), 16 (16.4%), 10 (10.3%) & 31 (31.9%) respectively. Rash, arthritis & bleeding manifestations were observed in 23 (23.7%), 8 (8.2%) & 21 (21.6%). Hepatomegaly, splenomegaly were seen in 86 (88.6%) & 71 (73.1%). Bicytopenia, pancytopenia were observed in 9 (9.2%), 26 (26.8%). Transaminitis, hypoalbuminemia & fulminant hepatic failure were observed in 48 (49.4%), 35 (36.08%), 4 (4.1%). Hemophagocytes on peripheral blood & BMA were present in 1 (1.03%) & 73 (75.2%). Hyperferritinemia, hyponatremia, hypertriglyceridemia & hypofibrinogenemia were observed in 82 (84.5%), 56 (57.7%), 31 (31.9%) & 35 (36.08%) respectively. Pigmentary dilution disorder, diagnosed on the basis of clinical phenotype, peripheral/BMA smear & hair mount &/or genetic testing, were recorded in eight (8.2%) children. Perforin analysis was performed for 76 children of which 9 (11.8%) were abnormal (average age at diagnosis of perforin deficiency-1.08 years). GRA was performed for 55 children of which 15 (27.2%) were abnormal (average age at diagnosis of GRA abnormality-2.47 years). MAS & IAHS were diagnosed in 8.2% & 14.4%, of which 6 children also had an abnormal perforin or GRA. Overall mortality of the cohort was 32.9% & mortality was as high as 66.6% in those with HLH due to perforin deficiency. Two relapsed after completion of treatment. None have received an HSCT to date.

**Conclusion:** HLH is a multisystem disorder. CNS involvement, as mentioned in literature, is seen in almost 30% of patients, which corresponds with this study. [1] Presence of hemophagocytes on BMA & hyperferritinemia are not universal findings. FHLH generally presents at an earlier age compared to secondary HLH & those who present as secondary HLH must also be tested for genetic defects. This study documented lesser perforin deficiency compared to published literature. [2] HSCT is a curative option for FHLH, but it is very difficult for children from developing countries to undergo timely HSCT.

(Ref: [1] Tae Woong Chung, *Korean J Radiol*, 2007. [2] C Gholam, *Clin Exp Immunol*, 2011)

Poster # 720

**DELAYED METHOTREXATE CLEARANCE IN CHILDREN, CAN WE PREDICT IT?**

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**Background:** Methotrexate (MTX) is a mainstay chemotherapy agent in protocols for Osteosarcoma and Leukemia treatment. The kidneys predominantly excrete methotrexate and decreased kidney function leads to prolonged exposure of the drug, increasing the likelihood of side effects and toxicity. Most protocols have rescue guidelines in place for leucovorin administration in the setting of delayed clearance; however there is currently no easy way to predict who will experience delayed clearance, acute kidney injury (AKI), or kidney failure. The pRIFLE criteria is a mechanism of defining children with AKI and may be a useful tool for determining risk of delayed clearance and therefore increased risk of toxicity.

**Objective:** To evaluate the association between AKI according to pRIFLE criteria and delayed methotrexate clearance.

**Method:** A retrospective chart review was performed on 128 Leukemia and 79 Osteosarcoma high dose methotrexate infusions from 2009-2014. Data was collected including creatinine values pre, during, and post MTX infusion, MTX dose, total clearance time, 24 hour MTX level (when available), and 48 hour MTX level. The Schwartz equation was used to calculate creatinine clearance and pRIFLE criteria were applied to each methotrexate dose visit to determine AKI. Statistical analysis was performed using t tests and linear regression analysis.

**Results:** Fifteen percent of osteosarcoma patients experienced delayed clearance of MTX, of which 25% met qualifications for AKI. The average age for osteosarcoma patients experiencing delayed and no delay in methotrexate clearance was 17.5 years and 14.5 years respectively ( $p < 0.005$ ). 46% of leukemia patients experienced delayed clearance of MTX, of which 3% met qualifications for AKI. The average age for leukemia patients experiencing delayed and no delay in methotrexate clearance was 12.7 years, and 8.9 years respectively ( $p < 0.005$ ).

**Conclusions:** While our study showed AKI classification using pRIFLE is of limited use in predicting delayed methotrexate clearance and further toxicity, it did show a significantly increased risk of delayed MTX clearance among older children. Further investigation will need to be performed to evaluate the true impact of age on methotrexate clearance as these findings may warrant closer attention to older children receiving methotrexate.

Poster # 721

## **VIRAL SURVEILLANCE USING PCR DURING TREATMENT OF AML AND ALL NOT INCLUDING HEMATOPOIETIC STEM CELL TRANSPLANT**

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**Background:** Polymerase Chain Reaction (PCR) based viral surveillance has become widely used in patients undergoing hematopoietic stem cell transplant (HSCT) and solid organ transplant. Disseminated adenovirus and cytomegalovirus (CMV) are potentially life threatening in immunocompromised patients. However, few studies have investigated the prevalence and morbidity of detectable viral replication and disseminated viral infections in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL) patients who have not undergone HSCT.

**Objectives:** The objective is to determine the incidence of detectable viral replication by PCR at

our institution where screening is performed for patients receiving intensive blocks of chemotherapy. Additionally, we will explore the clinical significance in those patients identified by PCR as at risk for disseminated viral infection and identify a subset of patients at the highest risk for clinically significant viral reactivation or infection.

**Design/Method:** We identified patients with newly diagnosed or relapsed acute leukemia at our institution between 2010 and 2014. Data excluded time points during or after hematopoietic stem cell transplant.

**Results:** The records of 118 patients with ALL and 31 patients with AML were reviewed. 66.9% of ALL patients and 100% of AML patients had at least 3 viral PCR tests and were therefore included in analysis. Of included patients, 36.4% had at least one positive PCR for adenovirus, CMV or EBV. 56.3% of viral detections were persistently positive. Intravenous immunoglobulin (IVIG) alone was given proximal to 31.0% of PCR positive events. Antiviral therapy was given as treatment during only 16.9% of PCR positive events, all in patients with ALL. In the ALL population, patients with high risk ALL, relapsed ALL or infantile ALL were more likely than patients with standard risk ALL to have a positive PCR identified.

**Conclusion:** Viral reactivation detected by PCR is common in this population, however, frequently self-limited. Current analyses are focused on identifying if risk factors such as age, immunoglobulin level (IgG), absolute lymphocyte count (ALC) or concurrent symptoms may be used to predict those at risk for clinically significant infection.

Poster # 722

## **PRIMARY BONE LYMPHOMA IN ADOLESCENTS AND YOUNG ADULTS TREATED WITH R-CHOP CHEMOTHERAPY**

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**Background:** Primary bone lymphomas (PBL) are rare. Treatment for adult PBL has been extrapolated from adult non-Hodgkin's lymphoma (NHL) trials. The optimal treatment of pediatric and adolescent and young adult (AYA) PBL is unclear and has followed pediatric Burkitt lymphoma intensive-timing regimen, or a nine week POG regimen, or adult NHL CHOP-based chemo-radiation regimen.

**Objectives:** To describe a series of AYA patients diagnosed with PBL treated with R-CHOP therapy at our institution.

**Design/Method:** A retrospective chart review of six patients treated in 2009 - 2012 was performed. Staging is defined as stage I: single osseous site; stage II: single osseous site with minor local lymph node involvement; stage IV: multiple osseous sites or local bone marrow involvement. "E" denotes extranodal disease. Following other published PBL series, stage III was not included in our definition.

**Results:** Based on disease response, patients received 4-8 cycles of R-CHOP and tolerated them well. Two patients received consolidative radiation (XRT) secondary to persistent PET-CT abnormalities. All patients are currently 2+ years from end of therapy with no evidence of disease (NED). (Table 1)

**Conclusion:** Response-based treatment with R-CHOP is effective and can be considered for AYA PBL. This outpatient regimen is well tolerated but greater than four cycles results in higher cumulative doses of anthracycline and alkylator chemotherapy as compared to lower stage Burkitt lymphoma regimen and nine week POG regimen. The POG regimen, also outpatient,



does not utilize imaging or histology to define complete remission. Whether a response-based treatment approach (PET-CT) is superior to historical treatments is unknown.

Table 1. Primary bone lymphoma in adolescents and young adults treated with R-CHOP

Patient	Gender	Age (years)	Site(s) of involvement	Stage	Number of RCHOP cycles	XRT	Follow-up (years)	Status
1	F	19	tibia	IE	6	yes	4	NED
2	M	19	tibia	IE	6	no	4	NED
3	F	20	femur, tibia	IVE	6	no	4	NED
4	M	21	femur	IE	6	yes	3	NED
5	M	17	Ilium with local marrow involvement	IVE	4	no	2	NED
6	M	15	Vertebral bodies, pelvis, tibia, fibula, femur, spleen	IVE	8	no	2	NED

Poster # 723

## CHARACTERIZATION, COST, AND OUTCOME OF BACTEREMIA IN CHILDREN WITH FEBRILE NEUTROPENIA AT A CHILDREN'S HOSPITAL

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**Background:** Febrile neutropenia (FN) is a potentially life-threatening complication of cancer chemotherapy in children. Few large-scale analyses have characterized the etiology, prevalence and outcomes of bacteremia in children with FN.

**Objectives:** To describe the prevalence, etiology, cost, and clinical outcomes of bacteremia during episodes of FN at our institution and compare to outcomes without documented bacteremia.

**Design/Method:** We performed a retrospective study of children with cancer admitted to Primary Children's Hospital (PCH; Salt Lake City, UT) with FN between January 1, 2009 and October 1, 2013. Our FN cohort was identified through the Intermountain Healthcare (IH) cancer registry and IH Enterprise Data Warehouse (EDW). An FN episode required an absolute neutrophil count (ANC) < 500, and blood culture obtained and/or administration of an anti-pseudomonal antibiotic. Clinical, laboratory and outcome data were electronically abstracted for each episode. Comparisons among patients with and without bacteremia were performed using regression models, with odds ratios (OR) and corresponding 95% confidence intervals (95% CI) reported for all statistical tests.

**Results:** 1,445 episodes of febrile neutropenia were identified in 511 children. In 951 (66%) cases, FN was the reason for admission. 176 (12%) of FN episodes had positive blood cultures. The most common pathogens identified were viridans streptococci (20%), *Escherichia coli* (13%) and *Pseudomonas aeruginosa* (8%). 12% of positive cultures had possible contaminants. 188 (13%) FN cases required PICU admission, of which 50 (27%) had blood cultures positive for presumed pathogens. Compared to children with negative blood cultures, children with bacteremia were more likely to require PICU admission (OR: 7.2 [95% CI: 5.1-10.3]), have a longer length of stay (OR: 2.5 [95% CI: 2.2-2.8]), have increased hospital costs (OR: 3.0 [95% CI: 2.5-3.5]), and die (OR: 4.9 [95% CI: 1.7-12.8]).

**Conclusions:** Overall, bacteremia was an infrequent occurrence in our large cohort of children with FN. Bacteremic children with FN experienced more serious clinical outcomes and greater financial burden than those without bacteremia. Viridans streptococci and gram negative organisms were the most common pathogens in bacteremic patients. Research improving

methods to discriminate between those at high and low risk of bacteremia could significantly improve patient care and lower healthcare costs.

Poster # 724

### **THROMBOTIC COMPLICATIONS OF PERIPHERALLY INSERTED CENTRAL CATHETERS DURING INDUCTION THERAPY FOR CHILDREN WITH ALL: A RETROSPECTIVE SINGLE INSTITUTION STUDY**

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**Background:** Children with acute lymphoblastic leukemia (ALL) require long term venous access for the delivery of therapeutic agents during the prolonged treatment course. Children with ALL are at increased risk of venous thromboembolism (VTE) due to the pathophysiology of disease, prothrombotic cancer-directed therapies, and the presence of central venous catheters (CVCs). Institutional variability exists in the timing and types of CVCs utilized during different stages of therapy for children with ALL. The most common complications of CVCs are VTE and central-line associated bloodstream infections. Over the past seven years, our institution primarily utilized peripherally inserted central catheters (PICCs) for induction therapy and has placed implantable ports following the completion of induction. PICCs may further predispose this patient population to thrombotic complications of CVCs.

**Objectives:** To describe rates of PICC-associated thrombotic complications in our ALL patient population during induction therapy and to compare these rates to those previously reported with other types of CVCs.

**Design/Method:** We performed a retrospective chart review to investigate the frequency of complications in children with ALL treated at Cincinnati Children's Hospital Medical Center (CCHMC) over the past seven years. Our analysis included those who initiated ALL therapy and were treated at least through induction therapy at CCHMC with sufficient data about diagnosis, treatment, and CVCs.

**Results:** One hundred fifteen patients received induction therapy for ALL at CCHMC between January 1, 2008, and October 31, 2014 and had sufficient data for review. Overall, 11 patients (9.5%) developed PICC-associated deep venous thrombosis during induction therapy. Data collection and analysis are ongoing for patient and treatment associated risk factors for PICC-associated thrombosis.

**Conclusion:** The use of PICC lines for ALL induction therapy is associated with significant morbidity. Compared to a meta-analysis investigating thrombotic complications for children with ALL the rate of VTE in our cohort during induction therapy (9.5%) is nearly double the rate reported for all children with ALL regardless of type of CVC (4.8%). Our data suggest that careful decisions must be made to weigh the risks and benefits of type of CVC.

Caruso V. et al, Blood, 2006

Poster # 725

### **Hodgkin Lymphoma Associated Paraneoplastic Syndromes**

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**Background:** Paraneoplastic syndromes (PNS) are rare disorders that are triggered by and altered immune system response to a neoplasm. A PNS may be the first or most prominent manifestation of malignancy at the time of diagnosis, and in many instances become the surrogate marker of disease recurrence. The association of PNS and lymphoma has been predominantly studied in adults affected with non-Hodgkin lymphoma. Few publications report on Hodgkin lymphoma (HL) and PNS particularly in children and adolescents. The pathophysiology of PNS is not known. It is speculated that immunoglobulin production is disturbed and that natural antibodies react with antigens of self-structures.

**Objectives:** To report our experience with 5 children and adolescents with HL and associated autoimmunity.

**Design/Method:** Case series

**Results:** Five patients with HL diagnosed at various institutions were found to have autoimmune disorders or PNS around the time of HL diagnosis (Table 1). The association of HL and PNS was established retrospectively once the diagnosis of HL was made. Most patients with HL and PNS had nodular sclerosis (NS) histology and advanced disease. The PNS was present 4 – 16 weeks prior to HL diagnosis. One patient was diagnosed with thrombocytopenia 3 years after diagnosis for HL, disease recurrence was not identified. As expected, PNS resolved after chemotherapy.

**Conclusion:** The association of HL and PNS is rare. Early recognition of the association of PNS and HL may help to diagnose HL earlier. The incidence of childhood HL associated PNS and the prognostic significance of PNS needs to be studied in a large cohort of patients.

Table 1. Characteristics of Patients with Hodgkin Lymphoma and Paraneoplastic Syndromes								
No.	Age of HL Diagnosis (years)	Histology	Stage	Treatment	Paraneoplastic Syndrome (PNS)	Time of Presentation of PNS in relation to HL diagnosis	Symptom/event Leading to diagnosis of HL	Resolution of PNS with chemotherapy
1	14	MC	IV-B (bulky)	ABVE-PC X 4 + RT	Nephrotic Syndrome	6 weeks prior	Cough and Chest XR after initiation of steroids	Yes
2	14	NC	II-B (Bulky)	ABVE-PC X4 + RT	Nephrotic Syndrome	8 weeks prior	Chest XR prior to start steroids	Yes
3	9	NC	IV-B (Bulky)	BEACOPP + RT	Eosinophilia (WBC 16,000/ $\mu$ L with 56% eosinophils) and Coombs positive test without hemolytic anemia	At diagnosis	Fever and cough Chest XR	Yes
4	14	NC	II-B (Bulky)	ABVE-PC x3 + RT	Hashimoto's Thyroiditis	16 weeks prior	Cervical adenopathy	No
5	18	NC	III	ABVE-PC x 5	Autoimmune Thrombocytopenia	3 years after diagnosis	Epistaxis	N/A

Poster # 726

## **IDENTIFICATION OF RISK FACTORS FOR HYPERSENSITIVITY REACTIONS TO PEG-ASPARAGINASE IN PEDIATRIC PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL)**

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**Background:** PEG-asparaginase is a pegylated formulation of *E. coli* L-asparaginase and is a standard component in the treatment of pediatric ALL. PEG-asparaginase preparations are bacterially derived, highly immunogenic and can cause severe hypersensitivity reactions. Prophylaxis for these hypersensitivity reactions may be ineffective and could decrease the efficacy of the drug. PEG-asparaginase has traditionally been administered intramuscularly (IM), but recently has been given intravenously (IV). There are conflicting data in the medical literature questioning the differences in hypersensitivity reaction rates between IV and IM administration of PEG-asparaginase. A recent publication by Petersen and colleagues found a significant increase in incidence of hypersensitivity reactions with the IV route compared to IM (19.5% vs 10.7%). Since the switch from IM to IV PEG-asparaginase in most protocols at the Cleveland Clinic, the incidence of hypersensitivity reactions in pediatric patients appears to have increased. If patients experience a hypersensitivity reaction to PEG-asparaginase they are switched to *Erwinia* asparaginase which is approximately ten times the cost of PEG-asparaginase per treatment course.

**Objectives:** The primary objective will determine the difference in hypersensitivity reaction rates between PEG-asparaginase IV and IM administration. The secondary objectives will describe the incidence and severity of hypersensitivity reactions and identify additional risk factors associated with hypersensitivity reactions to PEG-asparaginase.

**Design/Method:** This study is a retrospective chart review (approved by the IRB). Patients will be divided into two groups based on whether they experienced a hypersensitivity reaction to PEG-asparaginase. Data points collected include: demographic information, diagnosis/cell lineage, route of administration, risk stratification, IV flow rate, allergic reaction grade and onset of symptoms, concomitant medications, number of PEG-asparaginase doses, hospital or ICU admission, and *Erwinia* administration. Baseline characteristics will be analyzed with descriptive statistics and  $\chi^2$ . Potential confounders will be analyzed using logistic regression.

**Results:** Data points have been collected on 68 patients. Total of 15 patients (22%) had a documented hypersensitivity reaction to PEG-asparaginase. Data analysis is underway to determine differences in groups and identify risk factors for hypersensitivity reaction.

**Conclusion:** Once data analysis is complete we plan to contribute our findings to the body of medical literature. If differences in reaction rates exist based on route of administration we can adjust the standard route of administration in current and future pediatric clinical trials and treatment protocols.

Poster # 727

## **EFFECTS OF BODY MASS INDEX AT DIAGNOSIS ON RELAPSE RISK IN PEDIATRIC HODGKIN LYMPHOMA PATIENTS**

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**Background:** Rates of childhood obesity in the United States have risen dramatically in recent decades, with more than 31% of children currently classified as overweight or obese. This raises concerns about the effects of weight on outcomes for pediatric illness, including cancer. There is some prior evidence of poorer outcomes for both adult and pediatric patients who are overweight or obese with varied cancer diagnoses. To date, there are no studies investigating outcomes in overweight and obese children with Hodgkin lymphoma (HL).

**Objectives:** To characterize the effects of body mass index at diagnosis on risk of relapse in pediatric patients with HL.

**Design/Method:** We conducted a retrospective chart review of 101 pediatric Hodgkin lymphoma patients treated between 1980 and 2010 at Phoenix Children's Hospital. Clinical data, including disease stage, clinical outcome, time to relapse, BMI at diagnosis, treatment regimen, radiation therapy, as well as other clinical parameters, were abstracted from electronic and paper medical charts as well as survivor clinic records.

**Results:** None of the patient characteristics – sex, race, age, clinical risk level, or radiation status – were significantly associated with BMI. Patients with increased BMI at diagnosis had an increased unadjusted odds ratio of 1.58 (95% CI: 0.50-5.28) for relapse, but this was not statistically significant. Radiation therapy was associated with increased risk of HL relapse (P=0.004). There were no variables significantly associated with time to HL relapse. Kaplan-Meier curves of relapse-free survival time suggested a trend toward decreased long-term disease-free survival time in patients with higher BMI.

**Discussion:** The relatively small sample size for this study precluded demonstration of statistically significant differences in HL relapse risk or time to relapse between BMI groups. However, exploratory analyses suggested a trend toward increased risk for relapse and shorter long-term disease-free survival in patients with higher BMI, and these results merit further investigation in larger studies. Improving our understanding of how BMI affects pediatric cancer outcomes is an important step toward identifying patients at increased risk and determining how best to individualize treatment and monitoring plans for overweight and obese patients.

Poster # 728

## **A PILOT STUDY OF MITOXANTRONE IN COMBINATION WITH CLOFARABINE (MITCL) IN CHILDREN, ADOLESCENTS AND YOUNG ADULTS (CAYA) WITH REFRACTORY/RELAPSED ACUTE LEUKEMIA**

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**Background:** Despite excellent outcomes in pediatric ALL, 20-30% of patients relapse or become refractory to frontline therapies with subsequent CR2 rates of only 60%. For multiply relapsed patients, response rates remain around 40%. Novel combinations with improved complete response rates are needed. Mitoxantrone and clofarabine have proven effect in patients with ALL and AML and possible synergism in vivo with less drug resistance.

**Objectives:** To determine the maximal tolerated dose and overall response rate of clofarabine in combination with mitoxantrone as reinduction therapy for refractory/relapsed acute leukemia.

**Design/Method:** Patients 0-30.99yr old with ALL, AML or NHL in 1<sup>st</sup>, 2<sup>nd</sup> or 3<sup>rd</sup> relapse OR primary induction failure were given 1 to 3 cycles of clofarabine (escalating doses 20 to 40mg/m<sup>2</sup>/day) x 5days in combination with mitoxantrone 12mg/m<sup>2</sup>/day x 4days. Dexrazoxane was given prior to Mitoxantrone. Dose escalation was planned every 3 patients pending dose limiting toxicities. CNS prophylaxis was achieved with liposomal cytarabine. Patients were allowed subsequent cycles pending response and anthracycline exposure.

**Results:** To date 13 patients have been enrolled on the safety portion of this study. Age: median 17yrs (1-23yrs); 8 ALL (3IF, 3Rel1, 2Rel2), 4 AML (3IF, 1Rel2), 1 NHL (PD). We are currently enrolling at Dose Level 4 (Clofarabine 40mg/m<sup>2</sup>). There have been no unexpected Grade III/IV

toxicities or dose limiting toxicities. Nine of 11 (82%) evaluable leukemia patients achieved a CR after 1 cycle of therapy. Of these patients, 78% achieved MRD negativity (<0.1%). Two patients with relapsed ALL had no response. One patient with relapsed/refractory NHL had progressive disease after 2 cycles. One patient with AML is pending. Eight of 9 patients achieving CR went on to receive an allogeneic HSCT with continued remission at a median follow up time of 129 days (range 31-473).

**Conclusion:** The combination of clofarabine and mitoxantrone reinduction therapy for relapsed or refractory acute leukemia appears to be safe and well tolerated. Initial data from the first 13 patients enrolled is encouraging with an 80% CR/MRD negative rate and no dose limiting toxicities. Once the MTD or tolerable dose is reached, an extended Phase II Study will be initiated.

Poster # 729

## **A NEW PERSPECTIVE ON NOTCH INHIBITION IN T-ALL: PRO-DIFFERENTIATION EFFECTS THAT SYNERGIZE WITH DEXAMETHASONE**

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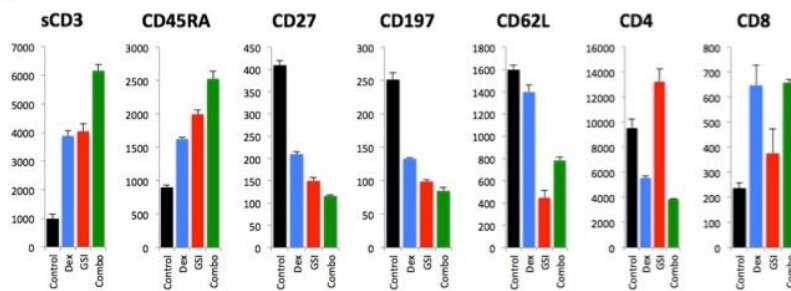
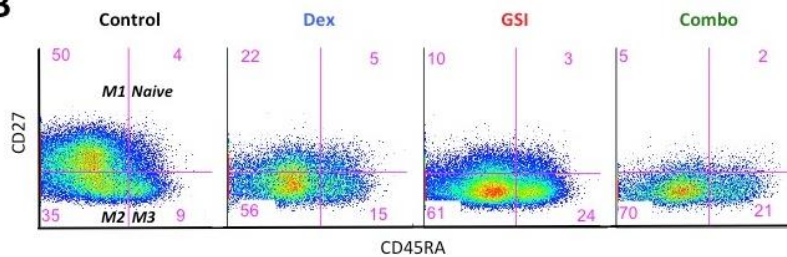
**Background:** Notch1 activating mutations occur in 50% of T-ALL, however clinical responses to Notch inhibition have been seen in both Notch1 mutated and unmutated T-ALL. We hypothesize that this activity may be due to a physiologic role of Notch in T cell differentiation. Indeed Notch signaling is down-regulated during the transition to mature T cells and constitutive Notch activation impairs this differentiation.

**Hypothesis:** We hypothesized that inhibition of Notch signaling may facilitate the differentiation of precursor T-ALL cells to more mature phenotypes, regardless of Notch1 mutation status.

**Methods:** Using human T-ALL lines (CEM, KOPTK1, HPB-ALL, Jurkat, MOLT4, SUPT1, and Loucy), gamma-secretase inhibitor (GSI, 1µM Cmpd E), dexamethasone (100nM) or the combination, we stained for T cell surface markers sCD3/4/8/27/45RA/197(CCR7)/62L.

**Results:** Treatment with GSI, dexamethasone or the combination, induced significant changes consistent with further maturation to memory-like cells in all seven T-ALLs. GSI significantly increased expression of surface CD3 (4 of 7 lines), CD8 (5/7), while decreasing markers of immaturity, CD62L (7/7), CD197 (4/7) and CD27 (5/7) (panel A). We observed shifts from early M1 to M2 and late M3 memory T cell phenotypes (panel B), suggesting GSI- and dexamethasone-induced differentiation and synergy.

**Conclusions:** We report the novel observation that Notch inhibition, via gamma secretase inhibitor, leads to the differentiation of T-ALL cells, regardless of whether they carry a Notch1 activating mutation. This may reflect a novel co-opted physiologic mechanism of T cell differentiation suggesting that Notch inhibition may have generalized activity in T-ALL, rather than only those with Notch1 activating mutations.

**A****B**

Poster # 730

## CANINES AND CHILDHOOD CANCER: WHAT ARE THE EFFECTS OF ANIMAL-ASSISTED INTERVENTIONS FOR PEDIATRIC ONCOLOGY PATIENTS AND THEIR PARENTS?

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**Background:** Although childhood cancer has profound psychosocial effects for children and families, few studies have critically evaluated complementary therapies to help families cope. Increasingly, attention has focused on the roles that animals play in supporting human health. However, while animal-assisted interventions (AAIs) occur daily in children's hospitals across the U.S., there remains a lack of rigorous research regarding their impact on patients, families, and therapy dogs.

**Objectives:** The *Canines and Childhood Cancer (CCC) Study* addresses these research gaps by rigorously measuring the effects of AAIs for pediatric oncology patients, their parents, and therapy dogs in multiple settings. Through human-animal interaction, CCC seeks to enhance childhood cancer treatment for patients and families through reduced stress and anxiety, and improved health-related quality of life.

**Design/Method:** Children, aged 3-17 years and recently diagnosed with cancer, and their parents are randomly selected to receive either their standard of care treatment only or their standard of care plus regular, 15 minute visits from a registered therapy dog and handler in the outpatient clinic. Both study cohorts participate for four months by completing psychosocial and behavioral instruments, such as the State Trait Anxiety and Pediatric Quality of Life Inventories, at designated intervals. Children also have their blood pressure and pulse measured at the beginning and end of each session. Additionally, therapy dogs have their session behavior videotaped and rated via handler self-reports and an AAI behavior ethogram on a weekly basis. Canine salivary cortisol is used to examine the dogs' stress levels during each AAI session, and is compared to their average baseline cortisol measurement, session behavior, and handler-rated temperament.

**Results:** Since 2014, 29 patients/families and 26 animal-handler teams have been enrolled.

Preliminary patient, parent, and canine findings will be presented, as well as lessons regarding successfully implementing AAIs in pediatric oncology settings. CCC data collection will conclude in late 2015.

**Conclusion:** CCC will advance the AAI and pediatric oncology fields through groundbreaking research, thus improving childhood cancer treatment and outcomes. CCC is supported by grant funding from Zoetis, the Pfizer Foundation, the Human-Animal Bond Research Initiative, and Morris Animal Foundation.

Poster # 731

## **RHABDOMYOSARCOMA OF THE BREAST IN ADOLESCENT AND YOUNG ADULT (AYA) WOMEN (A CASE SERIES)**

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**Background:** Soft tissue sarcoma (STS) constitutes 8% of all tumors in the adolescent and young adult (AYA) population, with rhabdomyosarcoma (RMS) accounting for only 5.2-6.5% of the STS total within this group. Compared to younger patients with RMS, AYAs have a higher propensity for metastasis as well as inferior outcomes. Common sites of metastasis include bone, lungs, lymph nodes, and bone marrow. Although rare, metastases to the breast have been reported in approximately 3-6% of RMS cases.

**Objectives:** Describe a single institutions experience with RMS and breast involvement, including the clinical presentation, patient characteristics, and outcomes.

**Design/Method:** A review of the Nationwide Children's Hospital Tumor Registry was performed and all cases of RMS diagnosed between January 1, 2004 and December 31, 2013 were identified. Data collected included age at initial diagnosis, histology of tumor, location of primary tumor and metastatic sites, treatment modalities, number of relapses, time to recurrence after initial treatment and survival time.

**Results:** A total of 46 patients with RMS were identified, having a mean age of 12.5 years (range 1-49 yrs). There were 26 males (57%) and 20 females (43%) total. Eighteen patients (39%) were in the AYA age range, including 10 women. In the total RMS cohort, four patients (8.7%) were identified with breast involvement at some point during their treatment, all of who were AYA females (constituting 40% of this subgroup). All 4 had disseminated disease at the time of breast involvement. One patient presented with primary RMS of the breast and 3 had involvement at relapse. Treatment modalities included chemotherapy, surgical resection, and radiation therapy. Only one patient is a long term survivor.

**Conclusion:** While rhabdomyosarcoma is uncommon in AYAs, breast involvement occurs almost exclusively in women of this age group and is associated with alveolar histology, metastatic disease, and poor outcomes. In this cohort, 40% of all AYA females had breast involvement at some point during their therapy. Routine imaging of the breasts in young women with RMS is not currently standard practice at diagnosis or follow-up, but this analysis suggests it should be considered in female AYA patients.

Poster # 732

## **EVALUATION OF FAMILY FUNCTIONING HEALTH RELATED QUALITY OF LIFE (HRQOL) IN CHILDREN WITH CANCER ON ACTIVE TREATMENT**



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**Background:** An understanding of the impact of having a child with cancer undergoing active treatment on family functioning helps to provide comprehensive care to families. There is limited knowledge about the scope of psychosocial and physical distress as measured by health related quality of life (HRQOL) evaluations in children with cancer on active treatment.

**Objectives:** To evaluate family functioning dimensions in children with cancer undergoing active treatment.

**Design/Method:** Parents or caregivers of children between the ages of 2 and 18 years with cancer participated in a cross-sectional study evaluating the impact of family functioning on HRQOL between June 2014-August 2014 at the Children's Hospital of Eastern Ontario. Parents were asked to complete the validated *PedsQL<sup>TM</sup> Family Impact Module*, which examines physical, emotional, social, and cognitive functioning, communication, worry, and the effect on daily activities and relationships.

**Results:** The *PedsQL<sup>TM</sup>* module was completed by 67 eligible families. The mean age of the children was 7.9 years. The mean time from diagnosis was 396 days, 67% of children were male, and 63% had a diagnosis of leukemia or lymphoma. There were negative HRQOL scores less than 50 points (on a scale of 0 to 100) in emotional, physical, social, and cognitive functioning in addition to having a negative impact on family relationships (Table 1).

**Conclusion:** The effect of family functioning on parents and families of children with cancer on active treatment is substantial. These results highlight the need for further psychosocial interventions for families of children with cancer on active treatment.

Table 1. Parental evaluation of family functioning measures of children with cancer on active treatment (N=67)

Dimensions of family functioning	Health related Quality of life Score, 0-100 (HRQOL) <sup>1</sup>
Physical Functioning	54.75
Emotional Functioning	49.5
Social Functioning	54.5
Cognitive Functioning	53.5
Communication	59
Worry	37.75
Daily activities	42.25
Family relationships	56.5
Overall average score of family functioning	46

<sup>1</sup>Higher scores indicate better HRQOL

Poster # 733

## COMPARISON OF CLINICAL CHARACTERISTICS AND OUTCOMES BETWEEN PEDIATRIC AND ADULT PATIENTS WITH DESMOPLASTIC SMALL ROUND CELL TUMOR

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**Background:** Desmoplastic small round cell tumor (DSRCT) is a rare, aggressive malignancy that typically affects pediatric and young adult patients. Due to its rarity, there are limited data on incidence and potential differences between pediatric and adult patients with DSRCT.

**Objectives:** Given the extreme rarity of DSRCT, we performed a population-based analysis using a large public registry with the following aims. First, we sought to better describe the overall incidence of this disease and to present detailed data on the incidence by age. Second, we sought to compare clinical features, treatment, and overall survival between pediatric and adult patients with this disease.

**Design/Method:** Patients aged 0-90 years at time of primary diagnosis with DSRCT and reported to the Surveillance, Epidemiology, and End Results (SEER) program between 1991 and 2011 were eligible for inclusion in the cohort. We estimated incidence rates by age. We used Fisher exact tests and log-rank tests to compare clinical features and overall survival for pediatric (age 0-21 years) and adult (age > 21 years) patients.

**Results:** The SEER database included 264 patients with DSRCT (95 pediatric / 169 adult patients). The median age was 25 years (range 0-90). The overall incidence rate across all ages was 0.16 cases/million person years, with a peak incidence rate in 20-24 year olds (0.36 cases/million person years). Compared to adult patients, pediatric patients were more likely to have pelvic tumors ( $p = 0.004$ ), have distant metastatic disease ( $p = 0.041$ ), and receive radiation therapy ( $p = 0.004$ ). Overall survival was poor and did not differ between pediatric vs. adult patients (5-year overall survival: 18.1% vs. 16.9%;  $p = 0.51$ ). Radiation therapy was associated with prolonged survival in pediatric but not adult patients.

**Conclusion:** DSRCT peaks in incidence during early adulthood. Clinical features and impact of radiation therapy differ between adult and pediatric patients. Outcomes for children and adults are uniformly poor.

Poster # 734

## IDENTIFICATION OF THE GENOMIC MUTATION THAT CAUSES A RARE INHERITED FORM OF KIDNEY CANCER

Marissa Leblanc, Amanda Brown, Daniel Gaston, Mathew Nightingale, Nancy Hamel, Jian Zhang, Emilie Lalonde, Jacek Majewski, William Foulkes, Andrew Orr, Conrad Fernandez, Christopher McMaster, Karen Bedard

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**Background:** Wilms Tumour is the most common type of childhood kidney cancer, caused by abnormal activity of renal stem cells. Most occurrences (95%) are sporadic, but familial cases do occur. Both sporadic and somatic mutations have been associated with mutations in the *WT1* gene. The Wt1 protein is involved in the development of the kidneys and gonads, playing a role in cell growth, cell differentiation and apoptosis. A familial version of Wilms Tumour has been identified where the *WT1* gene, and other known Wilms Tumour causative gene, was determined to have no mutations.

**Objectives:** Our project aims to find the causative genetic mutation of a hereditary version identified in extensive Atlantic Canadian kindred, where individuals do not possess mutations in known Wilms Tumour genes.

**Design/Method:** In an effort to find the genomic mutation that causes Wilms Tumour, genetic mapping by genome wide single nucleotide polymorphism (SNP) (Homozygosity Haplotyping)

was performed to identify regions of the genome that are shared between affected related individuals. The shared genetic regions identify areas of the genome where the causative mutation must lie. To identify the causative mutation within these shared regions, we have used 1) targeted sequencing of plausible genes; 2) whole-exome sequencing; and 3) whole-genome sequencing.

**Results:** As the causative mutation does not appear to be a simple nucleotide change within a coding region, we used SNP mapping data, Q-PCR and MLPA to investigate variations in DNA copy number as a possible cause. RNA-seq on samples isolated from patient kidney in tumorous and normal regions has led to the identification of abnormally expressed genes. Because clinical observation has identified that the age of disease onset differs depending on whether the mutation is inherited maternally or paternally, we are investigating DNA methylation, and therefore epigenetics, as a cause for this differential gene expression.

**Conclusion:** Success with this research project has the potential benefit of expanding the understanding of Wilms Tumour and may lead to the implementation of a DNA diagnostic tool that would facilitate the early identification of those individuals at risk.

Poster # 735

### **PRECLINICAL EFFICACY STUDY WITH BI-FUNCTIONAL SHORT-HAIRPIN RNA (bi-shRNA) FOR EWING SARCOMA**

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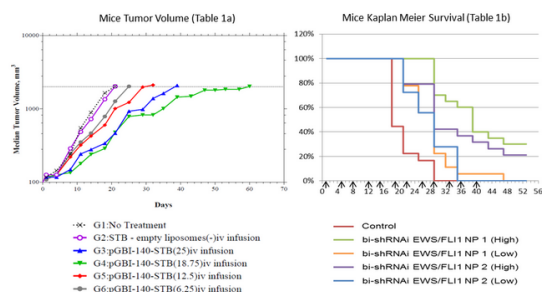
**Background:** Metastatic or recurrent EWS has a dismissal prognosis. High percentage of EWS tumors are characterized by a balanced chromosomal translocation t(11:22), in which the RNA-binding domain of the EWSR1 protein is replaced by the DNA-binding domain of the FLI1 protein. This creates an opportunity to develop a driver mutation specific target therapy.

**Objectives:** To develop a bi-shRNAi platform technology and delivery system to formulate a EWS targeted gene therapy.

**Design/Method:** We used a “bifunctional” RNAi strategy, concurrently inducing sequestration in the p-body (cleavage-independent) as well as post-transcriptional mRNA cleavage (cleavage dependent) mechanisms of translational repression to knock-down the expression of the EWS-FLI1 fusion protein. We tested EWS *in-vitro* cell lines and *in-vivo* mice model. The bi-shRNAi EWS-FLI1 vector was incorporated into 1, 2-dioleoyl-3-trimethylammonium-propane (DOTAP) and cholesterol for systemic delivery.

**Results:** Testing of the bi-shRNA EWS-FLI1 vector *in-vitro* revealed 80-92% knockdown of fusion gene and downstream translated protein (FLI-1 and CD-99 antibody) in a SK-N-MC cell line. Systemic administration of a Lipoplex formulation of bi-shRNA EWS vector in a SK-N-MC Ewing sarcoma mice model reduced tumor growth and increased survival ( $p < 0.005$ ) in a dose responsive manner without toxicity (Table 1).

**Conclusion:** This unique nanoplex bi-shRNAi EWS-FLI1 vector plus delivery vehicle satisfied preclinical efficacy, safety and biodistribution testing to justify a phase 1 clinical trial.



Poster # 736

## DIFFERENTIAL EFFECTS OF MXI1 AND MXI0 ON N-MYC-DEPENDENT NEUROBLASTOMA PATHOGENESIS AND CHEMOSENSITIVITY

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**Background:** Neuroblastoma is the most common extracranial malignancy of childhood. The Myc family of proteins regulates cell growth and proliferation and has been implicated in the etiology of many cancers. *MYCN* amplified neuroblastoma is associated with a poor prognosis. Investigating specific tumor pathways will further our understanding of neuroblastoma pathogenesis and lead to future therapeutic options. Mxi1 is a member of the MAD family that inhibits N-Myc activity. Mxi0 is an alternatively spliced variant of Mxi1 with a different first exon (Exon 0) whose function has not been determined.

**Objectives:** Test the hypothesis that Mxi1 and Mxi0 differentially impact N-Myc-dependent neuroblastoma cell proliferation.

**Design/Method:** We expressed Mxi1 and Mxi0 in SHEP neuroblastoma cells and SHEP cells stably transfected to express high levels of *MYCN* (SHEP/*MYCN*). We also utilized native neuroblastoma cell lines with inducible expression of Mxi1 and Mxi0. Cell proliferation and survival were quantified using BrdU and MTT assays. Apoptosis was measured by propidium iodide staining and caspase-3 immunohistochemistry. Subcellular localization of Mxi1 and Mxi0 proteins was detected by immunofluorescence.

**Results:** Overexpression of Mxi1 inhibits N-Myc mediated cell proliferation. In the absence of N-Myc, Mxi1 overexpression independently inhibits cell proliferation and induces cell apoptosis. Conversely, overexpression of Mxi0 in *MYCN* amplified neuroblastoma cell lines leads to enhanced proliferation, suggesting that Mxi0 has a counter-regulatory role to that of Mxi1. Compared with Mxi1, expression of Mxi0 results in cells becoming more chemoresistant. Finally, examination of Mxi1 and Mxi0 subcellular location reveals that Mxi1 resides in the nucleus while Mxi0 is found primarily in the cytoplasm; this differential localization appears to be determined by the presence of Exon 0.

**Conclusion:** Overexpression of Mxi1 in neuroblastoma cell lines leads to inhibition of N-Myc-mediated cell proliferation while Mxi0 appears to promote cell growth. Mxi1 expression enhances chemosensitivity of neuroblastoma cells, while Mxi0 has the opposite effect. Exon 0 may play an important role in the differential function. A better understanding of the interaction between Mxi1 and Mxi0 and how the balance of these proteins affect neuroblastoma physiology may aid in developing more effective targeted therapies to improve outcomes in children with neuroblastoma.

## TAXOTERE, AVASTIN, GEMCITABINE "TAG" CHEMOTHERAPY FOR HIGH RISK RELAPSED AYA SARCOMA PATIENTS.

Christopher Kuo, Paul Kent

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**Background:** Adolescent and young adult (AYA) patients with high-risk recurrent sarcomas have very poor prognosis, with few living 5 years. There are currently no standard treatments for these patients. Docetaxol (Taxotere,T) , Bevacizumab (Avastin,A), and Gemcitabine (Gemzar, G) have activity in sarcomas. One study of 3 children using "TAG" yielded a partial response in 2 patients and stable disease in one (JPHO 2012 34:524-7).

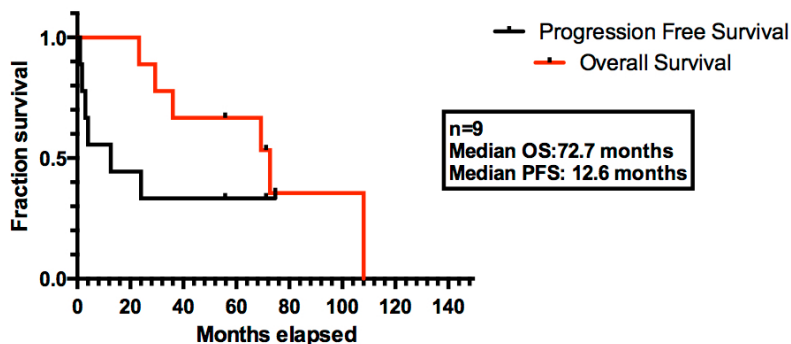
**Objectives:** We report our preliminary experience with this novel regimen.

**Design/Method:** From 2000-2011, 6 to 8 3-week cycles of TAG (T=100m/m<sup>2</sup> Day 8, A = 15mg/m<sup>2</sup> Day 1, G = 1,000 mg/m<sup>2</sup> Days 1 and 8) was given to 9 AYAs with relapsed sarcomas (3 had Ewings, 3 osteosarcoma, and 3 other). The median age was 29 (15-48), 5 were male, 6 had multiple relapses. All were pretreated with multiple chemotherapy regimens, 5 with multiple surgeries, and 2 with radiation.

**Results:** Median overall and progression free survival were 72.7 and 12.6 months respectively. Three patients achieved clinical remission, two had partial responses (70% and 40% reduction by RECIST), while 3 had progressive disease. Side effects included: mucositis, nasolacrimal duct obliteration, septal perforation, infection and thrombosis. 47 cycles of TAG were given with no toxic deaths, organ failure or need to stop prescribed therapy.

**Conclusion:** In our uncontrolled study, TAG showed response in 55% of relapsed, pretreated, high risk sarcoma patients with a majority living 5 years. TAG's toxicities were manageable. TAG may offer a choice to highly treated high risk AYA patients with sarcoma.

### TAG: Overall Survival (OS) and Progression Free Survival (PFS)



## MULTIMODALITY TREATMENT OF PEDIATRIC ESTHESIONEUROBLASTOMA

Rajkumar Venkatramani, Arnold Paulino, Carla Giannoni, Murali Chintagumpala, Josephine Haduong

**Background:** Esthesioneuroblastoma (EN) is a rare tumor arising from the olfactory neuroepithelium of the nasal vault. Surgery and radiation therapy are principal modalities used in adults with EN. However long-term consequences of these modalities pose unique challenges in children with EN.

**Objectives:** To describe the clinical presentation, diagnosis, treatment and outcome of patients diagnosed with EN in patients under 21 years of age.

**Design/Method:** Retrospective analysis of patients with EN diagnosed between 1990 and 2014.

**Results:** Seven children (aged 6 months to 15 years) were identified (Table 1). Complete resection of the tumor was performed in two children with Kadish Stage B at diagnosis while the other patients underwent upfront biopsy. Six patients received Ewing sarcoma type therapy, and very good partial response was achieved in four of the five patients with evaluable disease. Five patients received radiation therapy. Radiation therapy is planned in another patient. One patient experienced relapse in the cervical lymph node 10 months after diagnosis. She underwent high dose chemotherapy with autologous bone marrow transplant and has no evidence of disease at 55 months after diagnosis. All patients were alive at last follow-up. Endocrine dysfunction, frequent sinus infections, craniofacial abnormality, anosmia and teeth problems were seen in patients who underwent radiation therapy.

**Conclusion:** Given the excellent response to chemotherapy, careful consideration should be given when planning local control to minimize morbidity. One possible strategy may be to omit craniofacial resection and deliver lower dose radiotherapy (< 60 Gy) to patients who respond to chemotherapy.

Table 1. Clinical features and outcome

Pt. No	Age (yrs)/sex	Presenting symptom	Kadish Stage	Definitive surgery	Chemotherapy	Response to chemo	XRT	EFS (mo)	Sequelae
1	0.6/M	Incidental finding, epistaxis	B	Upfront CR	VDC/IE	NE	No	57	None
2	8/F	Headache, ptosis, ophthalmoplegia, Visual loss	C	Upfront biopsy	VDC/IE	VGPR	Nasopharynx- 5940 cGy Cervical- 5040 cGy	132	GH deficiency, Hypothyroidism, delayed puberty, frequent sinus infection, decayed teeth
3	14/F	Headache, epistaxis, hyposmia	C	Upfront biopsy	VDC/IE	VGPR	Pending	2	None
4	14/F	Nasal obstruction, facial swelling	B	Upfront CR	None	NA	Nasopharynx 5580 cGy	59	Craniofacial abnormality
5	10/F	Headache, dizziness	C	Delayed PR	VDC	SD	Nasopharynx 6120 cGy	54	Anosmia
6	15/M	Epistaxis, nasal obstruction	B	Upfront biopsy	VDC/IE	VGPR	Nasopharynx 5580 cGy	56	None
7	15/F	Headache, epistaxis, neck mass	B	Upfront biopsy	VDC/IE	VGPR	Nasopharynx 4500 cGy Neck 5000 cGy	10	None

XRT, radiation therapy; EFS, event free survival; OS, overall survival, V, vincristine; D, doxorubicin; C, cyclophosphamide; I, ifosfamide; E, Etoposide; NE, not evaluable; VGPR, very good partial response; GH, growth hormone.

## **PATTERNS OF FAILURE AND OPTIMAL RADIOTHERAPY TARGET VOLUMES IN PRIMARY INTRADURAL EXTRAMEDULLARY EWING SARCOMA**

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**Background:** Primary intradural extramedullary Ewing sarcoma (PIEES) is a rare tumor that is often treated with focal radiotherapy and systemic chemotherapy.

**Objectives:** To determine patterns of failure and optimal radiotherapy target volumes for PIEES.

**Design/Method:** The Mayo Clinic tumor registry was utilized to identify all Ewing sarcoma of the spine diagnosed after 1992, when Ewing translocation analysis was standardly performed. A retrospective chart review was performed to identify intradural extramedullary tumors. A PubMed search was performed using the keywords Ewing sarcoma, intradural, extramedullary and cauda equina. Only reports including radiotherapy dose and target volume, follow-up information and patients who received Ewing-like chemotherapy were included.

**Results:** Tumor registry review identified 25 patients with Ewing sarcoma of the spine, of which 4 patients had PIEES. All four patients were male, age 25 - 60 years old, and treated with vincristine, doxorubicin, ifosfamide, etoposide +/- cyclophosphamide. Two patients were treated with focal radiation to 50.4 Gy. Both recurred outside of the radiotherapy volume, but within the craniospinal axis at 4 and 41 months after completing therapy, and have subsequently died. One patient received craniospinal radiotherapy to 36 Gy with an additional 18 Gy to the primary disease, and is without evidence of disease at 20 months. The fourth patient is currently under treatment with a planned 30 Gy to the craniospinal axis and 59.4 Gy to the primary disease. Literature review identified 13 case reports that met criteria. Seven patients were male and 6 female, age 10 - 70 years old. All patients received focal radiation only, with total doses ranging from 30 - 56 Gy. Six of the 13 patients experienced distant craniospinal axis failure. Median time to recurrence was 7.5 months (range of 2-31 months). Three of the 6 patients with craniospinal recurrence died at 8, 14 and 48 months from diagnosis.

**Conclusion:** PIEES is a rare tumor with poor outcomes and appears to have a significant risk of craniospinal dissemination similar to central primitive neuroectodermal tumors. 8 of 17 patients treated with focal radiotherapy experienced distant craniospinal axis failure. Craniospinal radiotherapy should be considered to improve long-term outcomes in this rare tumor.

Poster # 740

## **EPIGENETIC THERAPIES IN PRECLINICAL MODELS OF NEUROBLASTOMA**

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**Background:** High risk neuroblastoma carries a poor prognosis despite intensive multimodal therapy, making the quest for novel therapeutic strategies an urgent need. A number of epigenetic abnormalities have been implicated in neuroblastoma. Recent studies suggest that epigenetic drugs require longer periods of exposure than cytotoxic drugs to exert their full effects, suggesting that conventional drug screening in monolayer cultures over 72-96 hours may be inadequate.

**Objectives:** The objective of our study was to identify combinatorial strategies targeting

epigenetic regulators for neuroblastoma. We used 3-dimensional cell culture systems to allow prolonged drug exposure durations.

**Design/Method:** A panel of “clinic-ready” epigenetic drugs, including vorinostat (histone deacetylase inhibitor), decitabine (DNA methyltransferase inhibitor), tranylcypromine (LSD1 inhibitor), EI1 (EZH2 inhibitor), JQ1 (bromodomain inhibitor), and EPZ00477 (DOT1L inhibitor), were screened *in vitro* in 3D cell culture conditions across a dose range from 0.01nM to 10µM. Cell proliferation was assessed following 12 days of exposure, dose-response curves were constructed, and IC<sub>50</sub> values were calculated using a variable Hill Slope model. Compounds with IC<sub>50</sub> values <10 µM were carried forward to assess combinatorial efficacy. For each pair-wise combination, all permutations of dose levels at tailored ranges bracketing respective IC<sub>50</sub> values were tested. Isobologram analyses were used to assess for additive, synergistic, or antagonistic effect.

**Results:** Vorinostat, decitabine, and JQ1 showed single agent efficacy *in vitro* with average IC<sub>50</sub> values of 600nM, 50nM, and 150nM, respectively, while tranylcypromine, EI1, and EPZ00477 were excluded for lack of efficacy. The combination of vorinostat+JQ1 revealed evidence of antagonism, whereas the combinations of decitabine+JQ1 and vorinostat+decitabine were consistent with additive effects.

**Conclusion:** While there is tremendous excitement about epigenetic therapies for cancer, there is a need to identify combinatorial strategies that enhance efficacy. Based on the current studies, we are assessing the *in vivo* efficacy of decitabine+JQ1 and vorinostat+decitabine in mouse models of neuroblastoma. Of note, the combination of vorinostat+decitabine is already in pediatric phase 2 trials for hematologic malignancies, thereby facilitating dosing information for potential clinical translation.

Poster # 741

## MYOFIBROMA IN INFANCY AND CHILDHOOD

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**Background:** Myofibroma is a rare fibroblastic-myofibroblastic tumor in children. Their biologic behavior is unpredictable and spontaneous regressions have been described.

**Objectives:** To describe the clinical characteristics, treatment and outcome of children with myofibroma from a single institution.

**Design/Method:** A retrospective analysis of children diagnosed with myofibroma between 1999 and 2013 was performed.

**Results:** The median age at diagnosis of 42 patients was 35 months. Approximately two-thirds of the patients were male (Table 1). The median follow-up was 48 months (range 0-165 months). Eighty-five percent of patients had solitary lesions. Nearly half of the tumors occurred in the head and neck, followed by trunk and extremities. 10 patients underwent a complete surgical resection. Of the 32 patients with positive or indeterminate margins, only one had tumor progression. One patient with generalized myofibromatosis involving bone and lung underwent a lobectomy at diagnosis. At 64 months follow-up she had stable or resolving bone lesions without further intervention. Another patient with progressive unresectable maxillary disease received neo-adjuvant chemotherapy (6 weeks of vincristine/dactinomycin/cyclophosphamide followed by 48 weeks of methotrexate/vinblastine), followed by complete surgical excision. All patients were alive at last follow-up.



**Conclusion:** Myofibromas of childhood may spontaneously regress and surgical resection or observation may be adequate treatment. The rare patient with progressive unresectable disease may benefit from chemotherapy.

**Table 1. Patient Characteristics (N=42)**

Age at diagnosis Median Range	35 months 7 days – 17 years
Length of follow-up in months Median Range	48 0 – 165
Sex Male Female	27 15
Location Head or neck Trunk Limbs Lung	20 10 14 1
Subtype Solitary Multicentric Generalized	36 5 1
Surgical Margins Negative Positive Unknown	10 26 6

Poster # 742

## THE IMPACT OF FGFR4 ARG388 ON NEUROBLASTOMA INCIDENCE, OUTCOME, AND RESPONSE TO TREATMENT

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**Background:** Children with high-risk neuroblastoma have poor outcomes. Improved understanding of the mechanisms underlying pathogenesis, recurrence, and treatment resistance will identify novel targets for future therapies. Aberrant growth factor receptor expression and tyrosine kinase signaling are associated with pathogenesis of many malignancies. These kinases are targets for novel therapies. A germline polymorphism in the *FGFR4* gene, (Arginine substituted for glycine at codon 388) is associated with decreased survival, treatment resistance, and aggressive disease in many malignancies.

**Objectives:** Determine the correlation of *FGFR4* genotype with neuroblastoma rates, outcomes and prognostic features, and determine the efficacy of FGFR4 inhibition in neuroblastoma preclinical models.

**Design/Method:** We screened DNA from neuroblastoma tumor cells and 129 patients for the *FGFR4* genotype using RFLP analysis. Allele frequencies were determined and compared to a matched control population. To evaluate the FGFR4 degradation rate, neuroblastoma cells were treated with ligand (bFGF) to induce receptor endocytosis. Western blots using antibodies against individual receptors were performed and relative expression levels were determined. We treated neuroblastoma cell lines with increasing concentrations of Ponatinib (a pan-FGFR inhibitor) and determined viability using MTT assays. Neuroblastoma cells were treated with either 5uM Ponatinib or untreated and observed for cell migration into a wound created in a monolayer of confluent cells. Cells were treated with 5uM Ponatinib and western blots using antibodies for downstream targets of FGFR4 were performed.

**Results:** Frequency of the A allele in neuroblastoma patients was increased compared to the

matched control population ( $p=0.014$ ). Logistic regression analyses suggested an association between having the "A" allele and having neuroblastoma, with a 3.3-fold increase in individuals with the AA genotype having neuroblastoma compared to individuals with the GG genotype. There was no difference in event-free or overall survival based on genotype. Ligand-induced FGFR4 protein degradation occurs slower than EGFR, consistent with increased FGFR4 stability. All neuroblastoma cell lines tested were sensitive to Ponatinib. Migration was inhibited and phospho-MEK and phospho-ERK were decreased after treatment with Ponatinib 5  $\mu$ M. **Conclusion:** The *FGFR4* Arg 388 polymorphism is associated with increased incidence of neuroblastoma. Treatment with Ponatinib is effective *in vitro* for treatment of neuroblastoma, potentially through inhibition of FGFR4.

Poster # 743

## **HSP27 AS A POTENTIAL REGULATOR OF GEMCITABINE-INDUCED AUTOPHAGY IN OSTEOSARCOMA CELLS**

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**Background:** Despite the different approaches to treat osteosarcoma (OS), pulmonary metastasis remains the main cause of death in these patients. Therefore, new therapeutic strategies are needed. We had previously reported that aerosol Gemcitabine (GCB) has a significant therapeutic effect against OS lung metastases. However, residual small isolated tumors, after GCB treatment, suggest a possible acquired resistance mechanism against GCB treatment. Autophagy, a catabolic process involved in cellular homeostasis, has emerged as an important mechanism involved in cancer cells response to treatment. Whether autophagy plays a role in OS resistance to GCB constitutes the basis of our studies.

**Objectives:** To investigate the role of autophagy in OS lung metastases resistance to GCB.

**Design/Method:** LM7 and CCH-OS-D human OS cells were treated with GCB and induction of autophagy was evaluated by western blot analysis, Acridine Orange (AO) staining and Electron Microscopy (EM) images. To test whether autophagy contributes to OS cells survival to GCB, we blocked autophagy by either knocking down Beclin (BECN) or by using a pharmacological inhibitor, hydroxychloroquine (HCQ), in combination with GCB.

**Results:** GCB induces autophagy in both of the OS cell lines tested as indicated by formation AVOs, conversion of microtubule-associated light chain 3 (LC3I/LC3II), increase in BECN and decrease in p62/SQSTM1 protein expression. Formation of autophagic vacuoles was also confirmed by electron microscopy. GCB-induced autophagy resulted in the inhibition of AKT /mTOR phosphorylation, suggesting this signaling pathway as a possible underlying mechanism responsible for GCB-induced autophagy. Sensitivity of LM7 cells to GCB was greatly enhanced after autophagy inhibition, suggesting autophagy as a pro-survival mechanism. Interestingly, inhibition of autophagy in CCH-OS-D cells decreased cell sensitivity to GCB, suggesting that GCB-induced autophagy contributed to cell death. Our preliminary work showed that the phosphorylation of Heat Shock protein 27 (HSP27) was significantly higher in GCB-treated LM7 cells when compared to CCH-OS-D GCB-treated cells, indicating a potential role of HSP27 in defining the role of autophagy in osteosarcoma cells.

**Conclusion:** Inhibition of GCB- induced autophagy can lead to either increase or decrease OS cells sensitivity to GCB. HSP27 may be an important factor to determine the fate of GCB-induced autophagy in OS cells.

## **UBE4B LEVELS ARE CORRELATED WITH NEUROBLASTOMA PATIENT OUTCOMES AND TUMOR DIFFERENTIATION**

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**Background:** UBE4B is an E3/E4 ubiquitin ligase whose gene is found in the chromosome 1p36 region commonly deleted in high-risk neuroblastoma tumors. We have previously observed a direct interaction between UBE4B and Hrs, a protein required for growth factor receptor (GFR) trafficking, suggesting a link between GFR trafficking and neuroblastoma pathogenesis. However, the functional roles of UBE4B in neuroblastoma tumor growth are not known.

**Objectives:** We analyzed the association of UBE4B protein expression with the outcomes of neuroblastoma patients and with neuroblastoma tumor prognostic features, GFR trafficking and downstream signaling.

**Design/Method:** We obtained neuroblastoma tumor tissue microarrays from the Children's Oncology Group and both formalin-fixed paraffin embedded (FFPE) tissue and fresh frozen tumor samples from the Texas Children's Hospital pathology department and tissue bank. We screened neuroblastoma tumor samples for UBE4B protein expression and for GFR expression and downstream signaling using immunohistochemistry and Western blots. Fluorescence in situ hybridization (FISH) for *UBE4B* and 1p36 deletion was performed on institutional tumor samples.

**Results:** In tumor tissue microarray samples, 26 of 43 differentiated tumor samples (61%) demonstrated increased UBE4B expression, compared to 24 out of 126 (19%) undifferentiated tumor samples. 17 out of 47 low stage neuroblastoma tumors (36%) and 3 of 4 stage 4 tumors post-treatment demonstrated increased UBE4B expression, compared to 2 of 21 (10%) untreated stage 4 tumors. Out of 29 institutional neuroblastoma tumor samples, FISH screening demonstrated *UBE4B* gene loss in all cases of 1p36 deletion. Reduction of UBE4B expression was only seen in poorly differentiated or undifferentiated neuroblastoma tumors or the poorly differentiated component of intermixed neuroblastomas.

**Conclusion:** We have demonstrated associations between *UBE4B* expression and neuroblastoma patient outcomes and between UBE4B and tumor cell differentiation in neuroblastoma tumor samples. In this study, reduced UBE4B expression in neuroblastoma tumors was associated with a lack of differentiation. Therefore, immunohistochemistry for UBE4B expression is a potential approach to address intratumoral heterogeneity in neuroblastoma. This link between a known cytogenetic risk factor, GFR trafficking, and neuroblastoma tumor differentiation suggests UBE4B-mediated GFR trafficking may contribute to the poor prognosis of neuroblastoma tumors with 1p36 deletions.

## **SUCCESSFUL CHEMOTHERAPY FOR PEDIATRIC PATIENTS WITH INFANTILE FIBROSARCOMA**

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**Background:** Infantile fibrosarcoma (IFS), a low-grade nonrhabdomyosarcoma soft tissue sarcoma (NRSTS), is the most common soft tissue sarcoma in children less than 1 year of age. These tumors most commonly present as a visible enlarging soft tissue mass involving distal extremities and complete conservative surgical resection is usually curative, impracticable thought. A number of reports have discussed the chemotherapy in the management of IFS.

**Objective:** We have described four children with IFS successfully treated with chemotherapy with or without conservative surgery.

**Design/Method:** This retrospective review highlights four cases of IF treated with chemotherapy with or without surgery to reduce the tumor at a single institution.

**Results:** Primary tumor site was the forearm (2), lower extremity (1) and the neck (1). All patients were started on a regimen of VAC chemotherapy followed by ifosfamide and etoposide. Response rate to chemotherapy was minimal. Although the dimension of these four tumors did not significantly change with chemotherapy, their radiographic appearances suggested some transformation had occurred. Therefore, resection rather than amputation was attempted and succeeded in three cases. All four are long-term survivors (range from 2 to 8 years) despite poor initial responses to treatment, or residual disease after treatment.

**Conclusion:** Chemotherapy can play a major role in patients with IF and initially unresectable tumors. Lack of radiographic response may not indicate a poor prognosis and attempts should be made to perform conservative, non-mutilating surgery to remove the tumor completely.

Poster # 746

## **WITHAFERIN A AS A NOVEL DIFFERENTIATING AGENT FOR TREATMENT OF NEUROBLASTOMA**

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**Background:** Neuroblastoma (NB), the most common extra-cranial solid tumor in children, originates from the precursor neuroblasts of the sympathetic nervous system. NB accounts for approximately 7-10% of childhood cancers and 15% of childhood cancer death. Despite an aggressive treatment regimen the prognosis for high risk NB remains poor. The differentiation of NB cells into mature cells represents a promising strategy for NB therapy. Currently retinoids are the most commonly used differentiating agents however their use can be limited due to intrinsic or acquired resistance as well as toxicity.

**Objectives:** To evaluate the potential of the natural product withaferin A (WA), a steroidal lactone derived from the medicinal plant *Withania somnifera*, to induce NB cell differentiation.

**Design/Method:** For differentiation studies NB cell lines (NB1691, SK-N-BE2C, SH-SY5Y and the primary cell line SVBM15) were exposed to WA (100-500nM) for 7-10 days and evaluated by light microscopy and western blot analysis. NB stem cell lines were generated by culturing NB1691 and SVBM15 cells in neurosphere media. To determine the IC<sub>50</sub> (concentration needed to reduce viability by 50%), NB stem cell lines were exposed to increasing concentrations of WA (0.1uM-10uM) and viability was assessed at 72 hours using MTS assay.

**Results:** WA promoted morphologic alterations (neurite outgrowth) and growth inhibition in a dose dependent manner. Western blot analysis indicated an increase in neuronal markers including neurofilament, vimentin, beta-tubulin and MAP2 and a decrease in the stem cell markers BMI-1 and musashi. WA induced NB stem cell death in a dose dependent manner. ( $IC_{50}$  of NB1691=1.05uM: SVBM15=1.06uM). Western blot analysis indicated that WA promoted endoplasmic reticulum (ER) stress as indicated by an increase in GRP78 and CHOP, and apoptotic cell death.

**Conclusion:** *Withania somnifera* has been used for centuries and is commonly used in ayurvedic medicine. WA has been shown to affect multiple pathways important for cancer progression and induce anti-cancer effects in breast, prostate and pancreatic cancers. Our data indicates that WA induces ER stress mediated NB stem cell death and promotes NB cell differentiation. WA holds promise as a novel treatment strategy for children with NB.

Poster # 747

## A NOVEL CpG ISLAND-FOCUSED ANALYSIS OF GENOME-WIDE METHYLATION PROFILING DATA IDENTIFIES EPIGENETICALLY DYSREGULATED GENES IN OSTEOSARCOMAS

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**Background:** Genome methylation profiling can identify gene targets of epigenetic dysregulation in cancers, but distinguishing biologically-relevant methylation changes from background 'noise' is challenging.

**Objectives:** To develop a robust genomic methylation profiling analysis to identify targets of aberrant epigenetic silencing in osteosarcoma.

**Design/Method:** Infinium HumanMethylation 450 Beadchip Methylation profiling quantitated 5-methylcytidine at 485,578 individual CpG sites in bisulfite-modified DNA samples of 16 primary human osteosarcomas. Data were reported as diff scores (D) reflecting difference in prevalence of methylation for each sample compared to pooled non-neoplastic controls (+D = gain of methylation and -D = loss of methylation) at each of the >480K individual CpG sites.

Analyzing only gene-associated CpG island sites, we hypothesized that genes exhibiting a disproportionately high site frequency of gain of methylation (+D) may be subject to biologically relevant epigenetic silencing. We therefore rank ordered CpG island loci along an observed/expected (Obs/Exp) hit distribution where: Obs = number of "hits" (where a hit is a CpG with  $D_{avg} \geq D_{Th}$ ) and Exp = # CpGs in the gene interrogated by the assay x (total # hits in the assay/total # CpG interrogated by the assay). A "difference threshold" ( $D_{Th}$ ) of 50 was chosen for this analysis.

**Results:** Of >15,000 CpG island genes interrogated at  $D_{Th} = 50$ , more than 2500 genes exhibited gain of CpG island methylation in excess of what would be expected based on a uniform distribution of CpG hypermethylation (Obs/Exp > 1). Of these, 169 genes exhibited hypermethylation at all CpG island sites assayed (Obs/Exp = 11.95). Among these were 14 genes with known or putative tumor suppressor function and 10 with cell cycle regulatory function. Eight genes encoded microRNAs previously shown to exhibit epigenetic dysregulation in cancer. Genomic regions exhibiting consistent CpG island hypermethylation included Chr19q13 (11 loci) and 16p11-12 (6 loci).

**Conclusion:** Genes exhibiting the highest Obs/Exp CpG island hypermethylation includes those

of potential significance to osteosarcoma tumorigenesis. The stringency of the screen may be varied by changing  $D_{Th}$ . The screen could potentially be made more robust by coupling with a relevant gene expression screen.

Poster # 748

## **DFMO INHIBITS NEUROSPHERE FORMATION AND TUMOR INITIATION IN NEUROBLASTOMA**

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**Background:** High risk neuroblastoma (NB) is characterized by MYCN amplification, age greater than 18 months, unfavorable histology, and aggressive metastatic disease. These patients can achieve remission but many relapse, with long term survival less than 10%. Prevention of relapse is critical. Polyamines are important in many cancers including NB and are inhibited through Ornithine Decarboxylase (ODC). High LIN28B expression is associated with worse survival outcomes and we recently published that Difluoromethylornithine (DFMO) targeting of ODC decreases LIN28 in NB. LIN28 overexpression is an important oncogenic driver in cancer stem cells which play an important role in relapse.

**Objectives:** To evaluate DFMO in targeting Cancer Stem Cells (CSC) via the ODC/LIN28/Let7 pathway to lead to reduced neurosphere formation and tumor initiation.

**Design/Method:** The neurosphere assay is used to isolate, expand and calculate the frequency of neural stem cells. Neurosphere assays were performed using DFMO (0.125-5nM) treated NB cell lines BE(2)-C, SMSKCNr, and patient derived NB cells which showed a dose dependent decrease in neurosphere formation. Evaluation of the tumor initiating efficiency was performed in serial dilution assays in mice xenografts treated with DFMO.

**Results:** *In vitro* experiments using NB cell lines show that DFMO treatment decreased neurosphere formation and tumor initiating ability in mice. At 5nM DFMO neurosphere formation was completely inhibited in triplicate assays ( $p < 0.01$ ). With the addition of increasing DFMO concentrations, there was a 9-fold decrease in percentage of neurospheres formed between 0.125 and 2.5 mM. A significant reduction of the tumor initiating efficiency in a serial dilution assay in mice injected with viable NB cells pretreated with DFMO for 10 or 20 days was observed.

**Conclusion:** Our previous studies have shown that DFMO affects these important cancer stem cell pathways. In addition, DFMO treatment of NB cells inhibits neurosphere formation suggesting that DFMO affects the cancer stem cells (CSC) ability to form neurospheres. This correlates with the mice xenograft studies in which DFMO pretreatment inhibited tumor formation in mice. DFMO therapy is currently in a clinical trial to evaluate its ability to target CSC in patients and prevent relapse in high risk NB.

Poster # 749

## **CHARACTERIZATION OF A NOVEL FUSION GENE, EML4-NTRK3, IN INFANTILE FIBROSARCOMA**

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**Background:** Classic infantile fibrosarcoma (IF) follows a benign course with a > 90% cure rate with surgery alone. IF is characterized by the fusion gene, ETV6-NTRK3, which portends a better prognosis. We describe a clinical case of a young child with a clinically aggressive presentation of IF and was found to harbor a novel chromosomal translocation, t(2;15)(2p21;15q25), resulting in the novel fusion gene EML4-NTRK3.

**Objectives:** To characterize the novel fusion gene, EML4-NTRK3, by assessing its *in vitro* and *in vivo* oncogenic potential.

**Design/Method:** Gene-specific primers of EML4 and NTRK3 were designed and the entire EML4-NTRK3 coding sequence was amplified by RT-PCR using total RNA extracted from primary tumor tissue. The EML4-NTRK3 sequence was subcloned into a lentiviral expression vector for subsequent viral transduction into NIH-3T3 cells. Following antibiotic selection of EML4-NTRK3-expressing clones, mock-infected and EML4-NTRK3-transduced cells were assayed for anchorage-independent growth via a soft agar assay and tumorigenic potential by subcutaneous injection into NOD-SCID mice.

**Results:** The cloned EML4-NTRK3 coding sequence was verified by Sanger sequencing and revealed in-frame fusion between exon 2 of EML4 and exon 14 of NTRK3. *In silico* analysis of the fusion sequence revealed the contribution of an oligomerization domain by EML4, and a kinase domain by NTRK3. Interestingly, we observed the loss of the NTRK3 transmembrane domain. Soft agar assay of EML4-NTRK3-transduced cells showed marked macroscopic colony formation in contrast to mock-infected cells which yielded no appreciable colonies. Subcutaneous injection of EML4-NTRK3-transduced cells yielded tumors (mean tumor volume of 2000 mm<sup>3</sup>; n=3/3 mice), and no appreciable tumor formation in mice injected with mock-infected cells.

**Conclusion:** EML4-NTRK3 is a novel fusion oncogene described from a patient with a clinically aggressive form of IF. *In vitro* and *in vivo* demonstration of the transformative potential of EML4-NTRK3 suggests that this novel fusion oncogene may be an important driver of malignant transformation in other cases of fusion-negative IF and, conceivably, other cancers. Future studies will evaluate the transformative potential of EML4-NTRK3 in human cell lines and characterize downstream targets of EML4-NTRK3 amenable to therapeutic targeting.

Poster # 750

## **RESPONSE AND RESISTANCE TO THE RECEPTOR TYROSINE KINASE INHIBITOR (TKI) VANDETANIB IN PEDIATRIC PATIENTS WITH MEDULLARY THYROID CARCINOMA (MTC)**

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**Background:** A phase I/II trial of vandetanib in children with advanced or metastatic MTC (NCT00514046) concluded that vandetanib 100 mg/m<sup>2</sup>/day is well tolerated and highly active.

**Objectives:** Report updated response data from this trial, and follow-up on patients who developed progression on vandetanib.

**Design/Method:** Patients 5-18 years old with measurable disease were eligible to enroll, and

received drug on a 28-day continuous dosing schedule. Responses were assessed using RECISTv1.0. Recommended dose, toxicities, and response data as of July 2011 have been previously reported. Patient charts were reviewed to update response data and provide follow-up on patients with disease progression on vandetanib.

**Results:** Of the 17 patients (9 male, age 9-17 years) enrolled on the trial between 7/2007 and 10/2012, 9 are currently receiving vandetanib on study. As of July 2014, median number of treatment cycles was 45 (range 2-84+). Best responses observed were: partial response (PR) 10/17, stable disease (SD) 6/17, primary progressive disease (PD) 1/17 (objective response rate 59%). Median cycle number to achieve best response was 20 (range 2-52, n=17) and to achieve PR was 10 (range 6-28, n=10). Six patients (3 with SD, 3 with PR) subsequently developed disease progression (cycle# 8, 28, 60 and cycle# 39, 44, 48 respectively). In 3 patients progression occurred in known disease sites; in 2 patients progression was observed in disease sites not identified on imaging at enrollment; in 1 patient progression was observed in the prostate, which in retrospect had evidence of metastatic involvement at enrollment. These 6 patients were subsequently treated with other TKIs - sunitinib: n= 3, sorafenib: n=1, cabozantinib: n=5 (4 on COG trial ADVL1211). Transient disease stabilization (10-16 months) was observed with sunitinib and sorafenib. The cabozantinib trial is currently ongoing.

**Conclusion:** Responses to vandetanib are sustained for prolonged periods in pediatric MTC. Complete responses are not observed, and several patients developed resistance after initial response or prolonged SD, 2 with progression in previously unidentified disease sites. Disease stabilization with other TKIs may be observed after progression on vandetanib. Molecular analysis from tumor tissue after disease progression is ongoing to explore mechanisms of resistance.

Industry support: AstraZeneca provided vandetanib for the trial

Poster # 751

## USE OF FISH AND FLOW CYTOMETRY FOR THE DETECTION OF MINIMAL RESIDUAL DISEASE IN EWING SARCOMA

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**Background:** Assessment of minimal residual disease (MRD) in blood or bone marrow may help monitor response to therapy and identify patients at greater risk for relapse. Both flow cytometry (FC) and fluorescent in situ hybridization (FISH) are used to measure MRD in leukemia patients, and we now describe these modalities for MRD assessment in Ewing sarcoma (ES).

**Objectives:** To describe the utility of FISH and FC for MRD detection in ES.

**Design/Method:** Peripheral blood (PB) and bone marrow (BM) samples were collected from patients at three pediatric cancer centers prior to, during, or following initial or salvage therapy for ES. BM samples were collected when clinically indicated, and PB samples were collected when patients were undergoing imaging assessment of disease. The FISH assay used a break-apart technique to detect tumor cells with *EWSR1* translocations, while FC identified CD99+/CD45- tumor cells. Morphology assessment by a blinded pathologist was also performed on bone marrow samples. Specimens were tested at a central laboratory via overnight delivery.



**Results:** Twenty-six patients with ES characterized by *EWSR1* translocations had 50 samples collected for testing by both FISH and FC, including 40 (80%) from PB and 10 (20%) from BM. No BM samples had disease identified by morphology or FISH. However, 4 of 10 BM samples at initial diagnosis were positive by FC, with one patient ultimately relapsing. Of PB samples, none were positive by both modalities, and 2 samples were positive only by FISH. Patients with same-day BM and PB testing often showed discordant findings with samples identifying tumor in BM but not PB in 4 patients by FC, and in PB but not BM in 1 patient by FC and 2 by FISH. Neither assay reliably detected MRD in the blood of patients with clinical or imaging evidence of disease progression.

**Conclusion:** Although some marrow samples were positive only by FC, the clinical significance was unclear. Neither FC nor FISH testing of PB consistently showed evidence of MRD, even in the setting of disease relapse/progression. The poor sensitivity of these assays may limit their clinical utility, and novel, more sensitive assays may be necessary in order to impact patient care.

Poster # 752

### **THE ROLE OF 18F- FLUORODEOXYGLUCOSE POSITRON EMISSION TOMOGRAPHY (18F-FDG PET) IN PEDIATRIC MEDULLARY THYROID CARCINOMA (MTC)**

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**Background:** Pediatric Medullary Thyroid Carcinoma (MTC) is a rare, relatively slow-growing cancer of the thyroid parafollicular C cells usually seen in association with the multiple endocrine neoplasia type 2 (MEN2) syndromes. The role of FDG-PET/CT in MTC is unclear but may assist in assessing disease activity.

**Objective:** To evaluate the role of FDG-PET/CT in assessing disease activity in pediatric MTC.

**Methods:** Patients  $\leq 25$  years with MTC and elevated calcitonin levels followed at the National Cancer Institute with  $\geq 1$  FDG-PET/CT were analyzed; the first available scan was reviewed with a nuclear medicine physician, who graded FDG-avid lesions as positive (likely/definitely related to MTC), questionable (possibly related), or negative (unlikely/unrelated) and reported maximal standardized uptake values (SUV). Anatomic imaging of disease sites was evaluated with RECIST, and the clinical course was obtained by chart review.

**Results:** FDG-PET/CT of 21 patients (ages 9 -21 years, 13 male, 20 MEN2B and 1 MEN2A) were analyzed. FDG-avid lesions were identified in the neck, mediastinum, lungs, liver, retroperitoneal nodes, and bones; no brain lesions were identified. In 4 patients, FDG-PET/CT was limited by significant brown fat. Ten patients had FDG-PET/CT while on treatment with tyrosine kinase inhibitors (TKIs) for MTC. Of these, 5 had  $\geq 1$  positive lesion (SUV 1.9 – 12.5), 3 had only questionable lesions (SUV 2.9–9.7), and 2 were negative. The 5 patients without positive lesions continued to show response by anatomic imaging and RECIST evaluation during 1-2 years follow-up, whereas 3/5 patients with positive lesions had disease progression on treatment within 7-10 months. Eleven patients had FDG-PET/CT while not receiving tumor-directed therapy. Of these, 5 had  $\geq 1$  positive lesion (SUV 1.0-8.6), 5 had only questionable lesions, (SUV 1.5-7.9) and 1 was negative. The disease course in these patients was variable.

**Conclusions:** FDG-PET/CT identified MTC-related lesions (maximal SUV  $<13$ ) in 10/21 patients. Of patients on TKIs, those responding had no FDG-avid lesions, suggesting that

FDG-PET/CT may have utility in monitoring response to therapy. However, the presence of positive lesions was not always associated with progression by RECIST. Longitudinal analysis in patients with  $\geq 2$  FDG-PET/CT and correlation to disease progression is ongoing to further understand the role of FDG-PET/CT.

Poster # 753

## **ALVEOLAR SOFT PART SARCOMA IN CHILDREN: AN INSTITUTIONAL EXPERIENCE**

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**Background:** Alveolar soft part sarcoma (ASPS) is a rare mesenchymal malignant tumor characterized by ASPL-TFE3 translocation. Apart from surgery, there is no standard management strategy for ASPS.

**Objectives:** To describe the clinical features, treatment and outcome of children with ASPS.

**Design/Method:** Retrospective analysis of patients with ASPS diagnosed at Texas Children's Hospital between 2000 and 2014.

**Results:** Nine patients (aged 10-21 years) presented with swelling and/or pain at the location of the tumor (Table 1). One patient presented with tongue bleeding. ASPS was confirmed by pathological examination. ASPL-TFE3 translocation was present in the five patients tested. The maximal tumor diameter was  $>5$ cm in seven patients. Complete surgical resection of the primary tumor at diagnosis was achieved in five patients. Delayed complete resection was achieved following tumor size reduction with sunitinib in one patient. Three patients received radiation therapy to the primary tumor bed after surgery. All five patients with localized disease were alive at last follow-up. Of the four patients with metastatic disease, two patients refused further treatment after diagnosis. One patient with multiple tiny biopsy proven lung nodules is being observed after primary tumor resection. The tumor was aggressive and unresponsive to multiple chemotherapy agents in one patient who died 15 months after diagnosis. Eight of the nine patients were alive after a median follow-up of 21 months.

**Conclusion:** Localized ASPS can be managed with surgery alone. There are no curative therapies for metastatic ASPS. Long term survival with indolent disease is possible in metastatic ASPS.

**Table 1. Clinical features and outcome**

Pt. NO	Age/ Sex	Location	Metastases	Surgery-primary	Chemotherapy	Length of follow up (months)	Vital status
1	10/F	Abdominal wall	None	CR	None	14	Alive NED
2	11/M	Left submandibular	None	PR	None	77	Alive with disease
3	16/F	Left thigh	None	CR	None	65	Alive NED
4	17/F	Tongue	None	Delayed CR	Sunitinib- 14 cycles- >70% reduction in tumor size	26	Alive NED
5	17/F	Right forearm	None	CR	None	13	Alive NED
6	14/F	Right forearm	Lung, breast, vertebrae	Biopsy	None	155	Alive with disease
7	16/M	Right thigh	Lung, vertebrae	CR	None	15	Alive with disease
8	9/M	Left hand	Lung	CR	None	6	Alive with disease
9	21/F	Thoracic paravertebral	Lung	PR	<u>Sorafenib</u> x 2 cycles- progression	15	Dead

CR, complete resection; PR, partial resection; NED, no evidence of disease.

Poster # 754

## MANAGEMENT OF CHILDHOOD BRONCHIAL CARCINOID TUMORS

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**Background:** Bronchial carcinoid tumors, although rare, are the most common primary malignant lung tumor in children. They are usually low-grade (typical) and treated with surgical resection

**Objectives:** To describe the clinical presentation, diagnosis, treatment and outcome of children with bronchial carcinoid tumors.

**Design/Method:** Retrospective analysis of clinical features and outcome of patients with bronchial carcinoid tumors diagnosed at Texas Children's Hospital between 1990 and 2014.

**Results:** Five children (aged 9 – 15 years) were identified (Table 1). Respiratory distress was the most common presenting symptom. One patient was diagnosed after lack of improvement of lung collapse after treatment for pneumonia. Another patient was treated for pneumonia and asthma for two years prior to diagnosis. Hemoptysis and Cushing syndrome (ectopic ACTH production in the tumor) were the other presenting symptoms. Computed tomography scan was used to identify the mass. Somatostatin uptake was present in the tumor in all three patients tested. Tumors were initially diagnosed by endobronchial biopsy. All patients had typical carcinoids and negative resection margin following definitive surgery. Lymph nodes were sampled in two patients and were negative for tumor. Bronchial stenosis requiring balloon

dilatation developed in one patient. Genetic testing for MEN-1 gene mutation performed in two patients was negative in both.

**Conclusion:** Typical bronchial carcinoid tumors in children are localized and amenable to curative surgical resection. Delay in diagnosis may occur when concurrent pneumonia develops due to obstruction.

**Table 1. Clinical features and outcome**

Pt. No	Age/ Sex	Presenting symptom	Location	Surgery	Duration of follow-up (mo)
1	9/F	Respiratory distress, wheezing	Left main stem bronchus	Left upper lobe sleeve lobectomy	7
2	11/M	Hemoptysis	Right main stem bronchus	Resection and reconstruction of right upper lobe bronchus	63
3	13/F	Tachypnea	Left main stem bronchus	Left lower lobe sleeve lobectomy	2.5
4	13/F	Facial acne, hirsutism, headaches, skin stretch marks	Right lower lobe	Wedge resection	2
5	15/F	Respiratory distress	Right lower lobe bronchus	Right middle and lower lobectomy	53

Poster # 755

## **SOLID TUMORS DURING INFANCY: A RETROSPECTIVE REVIEW OF CASES OVER AN 18-YEAR PERIOD IN A TERTIARY CENTER IN CENTRAL ILLINOIS**

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**Background:** Solid tumors (ST) are rare in children less than 1 year of age. Only 2% of childhood tumors are reported in the newborn period. However the actual incidence in this age group is difficult to determine. It has been speculated that the incidence of ST in neonates may be higher in rural/agricultural areas.

**Objectives:** To explore the epidemiology of ST during infancy (0-365 days) at our institution which serves a widespread rural population in central Illinois

**Design/Method:** Retrospective review of infants presenting with ST between 1996 to 2014 at the Children's Hospital of Illinois.

**Results:** Of the 991 pediatric patients diagnosed with tumors during the study period, 71 were infants. Ten infants with leukemia and histiocytosis were excluded from the analysis. The overall incidence of ST in infancy was estimated at 6% since 1996.

Of those, 17 patients (28%) were neonates (0-28 days) resulting in an overall incidence of 1.7%. Diagnosis was made antenatally in 7 patients (40%), or in the first 14 days of life in 10 patients (60%). Diagnoses in this group included neuroblastoma (n=4), muscular/connective tissue tumors (n=4), CNS tumors (n=3), hepatoblastoma (n=2), germ cell tumors (n=2) vascular tumors (n=2). The treatment modalities used in the neonatal group were observation (n=6), surgery only (n=4), chemotherapy (n=2), surgery and chemotherapy (n=5). Mortality occurred in 4 patients (25%). Forty four patients (72%) were diagnosed after the neonatal period (29 – 365 days) resulting in an overall incidence of 4.4% for this group. Diagnoses included neuroblastoma (n=17), CNS tumors (n=10), muscular/connective tissue tumors (n=5), hepatoblastoma (n=1),

germ cell tumors (n=4), renal tumors (n=6). The mean age at diagnosis was 7 months. The treatment modalities used were observation (n= 6), surgery only (n=13), chemotherapy (n=2), surgery and chemotherapy (n=21). Five patients succumbed to their disease (14%).

**Conclusion:** The incidence of ST during in infancy in the geographical area we serve was similar to that reported by other centers. Contrasting the incidence and epidemiological characteristics of ST between rural and urban populations may help elucidate the etiology of ST in Illinois.

Poster # 756

## **IDO-BASED IMMUNE CHECKPOINT BLOCKADE FOR PEDIATRIC BRAIN TUMORS: PRECLINICAL DATA AND CLINICAL TRIAL DESIGN**

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**Background:** Mounting evidence suggests that immune processes play a critical role in tumor clearance after standard treatments such as chemotherapy and radiotherapy. We hypothesize that responsiveness to these treatments is heavily dependent upon the responsiveness of a patient's immune system. The indoleamine 2,3-dioxygenase (IDO) pathway is a natural immune counter-regulatory mechanism that inhibits inflammation and immune responses. Unfortunately, in the setting of cancer, IDO suppresses desirable immune responses against the tumor. Drugs that block the IDO immune checkpoint pathway, such as indoximod (1-methyl-D-tryptophan), are in early-phase clinical trials for treatment of solid tumors, but until recently these trials have been limited only to adults.

**Objectives:** To summarize preclinical data and clinical trials using IDO-pathway blockade as adjunctive immunotherapy for the treatment of progressive pediatric brain tumors.

**Design/Method:** Preclinical data were generated using an established syngeneic glioblastoma model (orthotopic intracranial GL261), and key results were verified using a syngeneic melanoma model (subcutaneous B16F10). Mice were treated with or without IDO-blocking drugs combined with standard therapies, including temozolomide and radiation. Archival biopsy specimens, obtained at initial diagnosis, from pediatric patients with glioblastoma, medulloblastoma, and ependymoma were screened for IDO expression using immunohistochemical techniques.

**Results:** In animal tumor models we found strong IDO expression in tumors after chemo-radiation therapy, and we showed that blocking IDO during conventional chemo-radiotherapy elicits synergistic effects on survival, changing the nature of the tumor response and leading to widespread intratumoral complement deposition and local tumor necrosis. In human archival biopsy specimens, we found that IDO was expressed in a majority of pediatric brain tumors assessed (53%, 16/30), including glioblastoma (9/10), medulloblastoma (4/10), and ependymoma (3/10). This data has led to a Phase I clinical trial expected to open in mid-2015, sponsored by a Biotherapeutics Impact Grant from Alex's Lemonade Stand, to determine the safety and feasibility of combining indoximod IDO-blockade with standard temozolomide therapy or radiation for the treatment of children with progressive brain tumors.

**Conclusion:** Our data support the hypothesis that IDO-pathway blockade using indoximod is a clinically translatable strategy to enhance the anti-tumor effects of standard multimodal therapy in pediatric brain tumors by driving intratumoral inflammation and immune activation.

## **A PILOT STUDY COMPARING THE ADDITION OF OLANZAPINE OR APREPITANT IN AN ANTIEMETIC REGIMEN FOR HIGHLY EMETOGENIC CHEMOTHERAPY**

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**Background:** Chemotherapy induced nausea and vomiting (CINV) remains a problem in pediatric oncology. There is a need for new agents aimed at preventing CINV. Adult trials have shown olanzapine to be an effective antiemetic. Olanzapine has also been shown to be safe and well tolerated in children for psychiatric indications.

**Objectives:** We undertook a feasibility study comparing olanzapine and aprepitant as antiemetic regimens for pediatric patients receiving highly emetogenic chemotherapy (HEC). A secondary aim was to obtain preliminary data regarding the effectiveness and tolerability of both olanzapine and aprepitant.

**Design/Method:** Patients age 4-21 receiving two cycles of the same HEC were eligible. The study is a randomized crossover design. Patients received aprepitant in one cycle and olanzapine in the other cycle; both were administered with ondansetron and dexamethasone as multiple antiemetic agents are typically required for HEC. Patients and caregivers logged episodes of emesis, use of breakthrough antiemetics and daily nausea ratings, for days 1-5. To rate nausea caregivers used a visual analog scale (VAS) (1-100) while patients used the Baxter Retching Faces (BARF) pictorial scale (0-10), with a higher value indicating greater level of nausea. A complete response was considered no emesis and no use of breakthrough medications, measured as acute (Day 1) and overall (Days 1-5).

**Results:** A total of 14 patients (ages 7-18) have enrolled to date, 9 have completed both cycles, 3 are currently active, 1 withdrew after cycle 1 and 1 withdrew prior to cycle 1. The caregiver/patient log has a current return rate of 89.4% (17/19). The overall complete response rates are: olanzapine 40% and aprepitant 33.3%. The acute phase complete response rates are: olanzapine 70% and aprepitant 66.7%. Mean parent VAS nausea ratings are: olanzapine 15.3 and aprepitant 21.5. Mean patient BARF nausea ratings are: olanzapine 1.87 and aprepitant 1.95. Two patients experienced grade I agitation during olanzapine cycles, no other adverse events were reported.

**Conclusion:** Olanzapine was well tolerated and demonstrated promise as an effective antiemetic in pediatric patients. There were no significant adverse events. A larger trial comparing olanzapine and aprepitant appears feasible.

## **DISRUPTED NEURONAL NETWORK ACTIVITIES IN GLIOBLASTOMA MULTIFORME**

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**Background:** Glioblastoma multiforme (GBM) is associated with seizures in 20-50% of patients. Although some antiepileptics could attenuate frequency of seizures, there are no drugs to abort development of epilepsy.

Disrupting Acid Sensing Ion Channels (ASIC) increases seizure severity and duration, their activation trigger neuron firing and lead to seizures. While ASICs modulate seizures, their significance in gliomas is not clearly understood.

**Objectives:** GBM triggers molecular cascades to induce hyperexcitability in surrounding neuronal network via aberrant neuronal activation by means of overexpression of ASICs in peritumoral neuronal network, ultimately leading to development of seizures.

**Design/Method:** Female athymic nude mice were implanted in right hippocampus with  $1 \times 10^5$  human GBM U87MG cells. A 16 electrode microprobe was implanted in right hippocampus at same time. Local field potentials (LFP) were measured on days 2, 8 and 12 post-implantation. Frequencies for delta (0.1-3.9 Hz), theta (4-8 Hz), beta (13-20 Hz), low gamma (21-40 Hz), and 200-300 Hz (HFO), were analyzed. Seizure susceptibility was induced by injecting sub-convulsive doses of pentylenetetrazole-PTZ and graded using Racine's score. Mice were euthanized on day 28 and brains were collected for histology.

**Results:** GBM mice showed higher seizures severity (stage=2.1) compared with controls (stage=1.2) ( $p=0.01$ ) after PTZ. Spontaneous LFP recordings at different peritumoral sites showed high amplitude of epileptiform activity distal to the GBM before extensive GBM infiltration ( $p<0.05$ ). Frequency analysis from different bands showed that gamma and HFO increased in distal regions ( $p = 0.04$ ) at 8 and 12 days. Immunohistochemistry staining for ASIC-1, ASIC-2 and glutamate receptor subunit Nr2b expression in normal brain tissue, peritumoral and tumoral area showed increase in ASIC-1 and ASIC-2 in peritumoral area (1.29 and 0.87 /nucleus) compared with normal tissue (0.72 and 0.58 /nucleus) and tumor area (0.19 and 0.06 /nucleus). Nr2b had similar levels in peritumoral and control.

**Conclusion:** GBM induces modification of neuronal network which mediates neuronal hyperexcitability and seizure susceptibility. We postulates that increase in ASICs and decrease of Nr2b unbalance the physiological neuronal network promoting hyper-excitability. Understanding the evolution of disruption of neuronal network could provide new target for intervention against epilepsy in gliomas.

Supported by grant from Hyundai Hope on Wheels

Poster # 759

## **TRENDS IN END-OF-LIFE CARE IN PEDIATRIC HEMATOLOGY, ONCOLOGY, AND BONE MARROW TRANSPLANT PATIENTS**

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**Background:** Early discussion of goals of care, including introduction of palliative care, increases patient/family satisfaction and improves quality of life. Both ASCO and AAP advocate for palliative care in all patients with life-threatening, complex illness, specifically cancer.

**Objectives:** To evaluate trends over time in end-of-life care services delivered to pediatric hematology/oncology/bone marrow transplant (BMT) patients.

**Design/Method:** A retrospective chart review of a single tertiary-care pediatric institution from 1/1/2002 to 3/1/2013 identified 445 evaluable hematology/oncology/BMT patients who died

during this interval. Categorical data and continuous data were analyzed using chi-square, t-test, and ANOVA, respectively, and assessed over quartiles for trends.

**Results:** Age at diagnosis ranged from 2 days to 25 years (mean 8.4 years); 63% (282/445) had at least one relapse, 45% (201/445) enrolled in hospice (median 28 days), and 11% (48/445) had a palliative care consultation. Oncologic diagnosis was associated with palliative care consultation ( $p = 0.046$ ) and hospice utilization ( $p < 0.001$ ), with neuro-oncology and solid tumor patients enrolled in hospice more frequently than leukemia/lymphoma patients (78% and 67% vs 33%, respectively). Palliative care consultation was associated with having a Do-Not-Resuscitate (DNR) order ( $p = 0.009$ ), but not with length of time from DNR to death or hospice utilization. Patients receiving Phase I therapy were enrolled in hospice more frequently ( $p < 0.0001$ ), for a longer time ( $p = 0.021$ ), and were more likely to die at home/inpatient hospice than in the hospital ( $p = 0.003$ ). Christian/Catholic religious affiliation was associated with increased hospice enrollment ( $p = 0.034$ ), but less days in hospice ( $p = 0.001$ ) compared to other religious affiliations. When patient deaths were analyzed over quartiles spanning 2002-2013, the frequency of DNR orders ( $p = 0.016$ ) and palliative care consults ( $p < 0.0001$ ) increased over time. Hospice enrollment, death location, and Phase I enrollment did not significantly change.

**Conclusion:** Despite statistically significant increases in palliative care consultation and implementation of DNR orders over time, utilization remains suboptimal. Additionally, there has been no increase in hospice enrollment or shift in death location. These data will help target future initiatives to achieve earlier discussions of goals of care and increased palliative care involvement for all patients.

Poster # 760

## THE USE OF COMPLEMENTARY AND ALTERNATIVE MEDICINE (CAM) IN CHILDREN WITH BRAIN TUMORS

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**Background:** CAM including natural products, mind-body therapy or dietary restriction is frequently used in cancer patients but is not often reported to health care providers. There is limited evidence based research that looks at the prevalence of CAM in pediatric oncology patients, especially in brain tumor patients.

**Objectives:** To find out the prevalence of CAM use in pediatric brain tumor patients

**Design/Method:** Questionnaires regarding CAM use were handed out to parents of children diagnosed with brain tumors on treatment or follow up at our institution. Usage of various forms of CAM was correlated with patient's age, gender, ethnicity, insurance, diagnosis, treatment modalities, year of diagnosis and body weight using Fisher Exact Probability test.

**Results:** Among the 72 respondents, 53 patients (73%) used one or more forms of CAM. 36 (49%) used natural product(s), 42 (58%) used Mind-Body therapy and 20 (27%) used a special diet or dietary restriction. CAMs are more likely used by Caucasians ( $p=0.016$ ) and patients treated with radiation therapy ( $p=0.013$ ). Mind-body therapies are more likely used by Caucasians ( $p=0.048$ ), parents with a college or graduate degree ( $p=0.006$ ) and diagnosis after 2010 ( $p=0.027$ ). Natural products are more likely used by patients with CNS Embryonal tumors ( $p=0.014$ ), patients who have received radiation ( $p=0.011$ ) or underweight patients ( $p=0.044$ ). The most commonly used natural products are antioxidant/fish oil (39%), mushroom extract (22%), Vitamin D (22%) and melatonin (16%). The most commonly practiced dietary



restriction is low sugar/Atkins/Ketogenic diet (80%). The most common Mind-Body therapy used is prayer (67%), followed by acupuncture (43%) and message therapy (40%). The most common goals for use of CAM are to boost the immune system (32%) and improve sleep (32%), followed by pain control (28%), stress reduction (28%), decrease nausea and vomiting (28%) and to decrease fatigue (19%). 22% of families spent more than \$100 per month on either natural products or mind-body therapy. 18% patients are under care of integrative medicine providers. 64% of parents informed oncology providers about use of CAM in their children.

**Conclusion:** In conclusion, CAM use is common in children with brain tumors and Mind-Body therapy is the most common type of CAM used.

Poster # 761

## **PHASE 1 EVALUATION OF EVEROLIMUS (MTOR INHIBITOR) IN COMBINATION WITH VANDETANIB (MULTIKINASE INHIBITOR OF EGFR, VEGFR, AND RET) OR VEMURAFENIB (BRAF INHIBITOR) IN CHILDREN AND ADOLESCENTS WITH ADVANCED CANCERS**

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**Background:** Early phase clinical trials are often limited to adults, reducing opportunity to explore safety and efficacy of new agents in children. Preclinical models have shown that concurrent inhibition of mammalian target of rapamycin (mTOR) pathway signaling cooperates with receptor tyrosine kinases to overcome primary and/or acquired resistance to tyrosine kinase inhibitors.

**Objectives:** Hypothesizing that mTOR inhibition may improve efficacy of multi-kinase targeting of BRAF, EGFR, VEGFR, we evaluated the combination of everolimus with multi-kinase inhibitor, vandetanib (NCT01582191), in patients with advanced malignancies and the combination of everolimus with BRAF inhibitor, Vemurafenib, in patients with BRAFV600 mutation (NCT01596140). Since these regimens may hold promise for young patients, children and adolescents were eligible for enrollment.

**Patients and Methods:** These phase I dose escalation trials were designed with a conventional “3+3” design. Study drugs are given continuously for 28 days. Endpoints included maximum tolerated dose (MTD), dose limiting toxicities (DLT), safety, and response using RECIST. CLIA certified next generation sequencing based molecular profiling was performed whenever feasible. Younger patients were enrolled at the accruing dose level, with BSA-based dose adjustments for smaller children.

**Results:** To date, 11 patients younger than 21 years of age (Vandetanib + everolimus, n=9; Vemurafenib + everolimus, n=2), mean age 14.7 (range, 10-20) years, with median of 3 (range 0-6) prior therapies have been enrolled. DLTs in children included grade (G) 4 thrombocytopenia at dose level 4 on the vandetanib trial and G3 rash on the vemurafenib trial (dose level 2). Of 8 evaluable patients on the vandetanib trial, 3 (37%) had stable disease: one patient with AKT1 p.E17K mutant pseudopapillary pancreatic tumor, one with ASPL/ASPSCR1-TFE3 fusion positive/argininosuccinate synthetase-1 negative alveolar soft part sarcoma, and one with osteosarcoma. A 10 yr old patient with BRAF V600E mutant pleomorphic xanthoastrocytoma had a partial response (32% decrease in tumor size) with vemurafenib/everolimus.

**Conclusion:** The DLT's of the combinations of everolimus with vandetanib or vemurafenib seen in children appear to be similar to those seen in adults. Early signals of activity were noted in heavily pre-treated children with refractory solid tumors. Including younger patients in institutional phase I trials provides a mechanism to study new combinations in children.

Poster # 762

## **OUTCOME OF PEDIATRIC PATIENTS WITH INTRASPINAL LOW-GRADE ASTROCYTOMAS**

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**Background:** Low-grade astrocytomas are the most common central nervous system tumors in the pediatric population, and are often difficult to treat with a response rate to therapy for brain astrocytomas of 60%. We sought to determine if the response rate to therapy for spinal cord astrocytomas was higher than for those in the brain.

**Objectives:** To determine the response rate of pediatric patients with low-grade astrocytomas in the spine compared to those in the brain.

**Design/Method:** A retrospective review of 50 patients with low-grade brain or spinal cord astrocytoma treated at our institution from 2000 to 2013.

**Results:** Five patients were diagnosed with low-grade spinal astrocytoma and 45 with low-grade brain astrocytoma. The mean age at diagnosis was 6.7 years and 8.3 years for spinal and brain astrocytoma, respectively. Thirty-nine (78%) patients underwent surgical resection and 16% underwent biopsy only. Of these, one spinal patient and 16 brain patients underwent complete resection, and 2 and 20, respectively, underwent partial resection. Of those with a partial tumor resection (56.4%), 31.8% and 4.5 % received adjuvant chemotherapy and radiotherapy, respectively. 14% of all patients received chemotherapy, without surgical resection. Of the patients with spinal tumors, those with a subtotal resection or biopsy only (n=3) received chemotherapy and showed a mean tumor decrease of 36.9%, and none relapsed. The 2 not treated with chemotherapy initially showed tumor progression at a mean of 29.5 months and subsequent chemotherapy resulted in tumor decrease or lack of tumor evidence. Of the 45 patients with brain tumors, 10 received chemotherapy and 4 received radiation therapy. Tumor size decreased by a mean 44.5% for those with surgery and adjuvant therapy, and 27.1% for those with adjuvant therapy alone. There were 10 patients who relapsed at a mean of 9.6 months. Those treated underwent resection (n=4) or adjuvant therapy (n=4). All of the latter and one of the former relapsed again.

**Conclusion:** All patients with low-grade astrocytomas of the spine had a response to adjuvant chemotherapy and none received radiation therapy, while only 81.8% of those with astrocytoma of the brain had a response to adjuvant therapy including radiation therapy.

Poster # 763

## **THE ROLE OF PET/CT VS BONE MARROW BIOPSY IN THE INITIAL EVALUATION OF BONE MARROW INFILTRATION IN VARIOUS PEDIATRIC SOLID TUMORS**

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**Background:** Accurate staging of pediatric solid tumors is essential in management and prognosis. The presence of bone marrow infiltration (BMI) is associated with higher international prognostic index (IPI) scores, more aggressive disease, and less favorable prognosis. The gold standard test to screen for BMI is bone marrow biopsy (BMB), and for convenience, the iliac crest is the site of choice. To complete the staging evaluation, radiological imaging is used, with positron emission tomography-computer tomography (PET/CT) being the most common modality. However, all pediatric trials require a BMB for assessment of BMI.

**Objectives:** To assess the role of PET/CT in comparison to BMB in the initial evaluation of BMI in various pediatric solid tumors.

**Design/Method:** A retrospective study at Miami Children's Hospital evaluated new oncology cases between January 2009 and October 2014 with the diagnosis of: Ewing's sarcoma, neuroblastoma, lymphoma, and rhabdomyosarcoma. Inclusion criteria required the case to be a first time diagnosis, to have PET/CT and BMB completed, and testing performed within 4 weeks.

**Results:** A total of 69 patients were eligible, 35 of which were considered non-metastatic by either modality. BMI was demonstrated in 34 patients by PET/CT and 18 by BMB. The sensitivity and negative predictive value of PET/CT were both 100%. Interestingly, several cases with negative BMB and an abnormal PET/CT showed focal BMI at the iliac crest or disease at other bone marrow sites (i.e. femur, humerus).

**Conclusion:** This study demonstrates that PET/CT has a high sensitivity when assessing BMI in various solid pediatric tumors. The use of the gold standard (BMB) should be reconsidered in cases where PET/CT does not depict BMI, as BMB is known to be invasive, painful, costly, and poses risks for infection and bleeding. Furthermore, BMB results are limited by insufficient tissue or degree of BMI (diffuse vs. focal disease). PET/CT can improve precision of biopsy when used as a guiding tool to sample the concerning bone marrow sites. Therefore, this study suggests PET/CT is a better screening study for BMI, as it improves accuracy of staging and stratification of high-risk cases.

Poster # 764

## **SOX2 Expression in Pediatric Brain Tumors: A Potential New Target for Immunotherapy**

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**Background:** Pediatric brain tumors remain a major cause of morbidity and mortality and new treatment approaches are needed. SOX2, an embryonal stem cell antigen important for maintaining pluripotency, is highly expressed in adult glioblastomas, especially within the stem cell population. We have previously shown that T-cell immunity to SOX2 can be detected in the peripheral blood of patients with cancer and that its presence correlates with response to checkpoint blockade therapy. We have also recently shown that T-cell immunity to SOX2 can be generated using dendritic cells loaded with SOX2 nanoparticles.

**Objectives:** The purpose of this study was to understand SOX2 expression in pediatric brain

tumors and to detect the presence of naturally occurring SOX2 specific T-cells in these patients.

**Design/Method:** We analyzed SOX2 expression using immunohistochemistry in archived tissue from 35 pediatric brain tumor specimens. In 5 patients, we were able to study the presence of SOX2 reactive T-cells in the peripheral blood using CXCL10 secretion assay using a peptide library created from the entire length of the SOX2 protein as previously published. In this assay, the peptide-induced secretion of CXCL10 served as a marker of SOX2 specific T-cell reactivity and values  $\geq 2$ -fold over the negative control were deemed positive for the presence of SOX2 specific T-cells.

**Results:** SOX2 was consistently expressed in pilocytic astrocytoma (14/14), grade III astrocytoma (3/3), and glioblastoma (5/5) and ependymomas (3/3). There was also frequent SOX2 expression in oligodendrogliomas (3/5) but no expression in medulloblastoma (0/5). Out of 5 pediatric brain tumor patients (3 pilocytic astrocytoma, 1 ependymoma, 1 oligodendroglioma), 1 patient with a pilocytic astrocytoma displayed naturally occurring T-cell immune response to SOX2.

**Conclusion:** SOX2 is highly expressed in pediatric glial tumors and naturally occurring SOX2 specific T-cells were detected in the setting of pilocytic astrocytoma, a subtype typically associated with an excellent outcome. SOX2 may therefore serve as an important immunotherapy target in pediatric glial tumors. Studies on the immunotherapeutic potential of SOX2 and strategies to augment the T-cell immune response through the use of nanoparticle-targeted dendritic cells and immune checkpoint blockade are warranted.

## THE GENETIC FINGERPRINT OF SUSCEPTIBILITY TO TRANSPLANT ASSOCIATED THROMBOTIC MICROANGIOPATHY

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**Background:** Transplant associated thrombotic microangiopathy (TMA) is frequent after HSCT, and in severe cases causes significant morbidity and mortality. Currently there are no data addressing individual susceptibility to transplant-associated TMA.

**Objectives:** Our study objective was to determine genetic predisposition to TMA and to better understand the pathogenesis of this severe HSCT complication.

**Design/Method:** Seventy seven consecutive allogeneic HSCT recipients were enrolled on a prospective TMA biomarker study and categorized as having TMA or no TMA using rigorous diagnostic criteria (Jodele et al, Blood 2014). Genomic DNA was isolated from pre-HSCT recipient blood. We used a candidate gene approach to identify 12 genes within the complement pathway likely to play a role in terminal complement activation, the likely effector mechanism for vascular damage in TMA. All exons, flanking intronic and untranslated regions of *ADAMTS13*, *CFH*, *CFI*, *CFB*, *MCP*, *THBD*, *C3*, *C5*, *CFD*, *CFHR1*, *CFHR3* and *CFHR5* were sequenced and reads were aligned against the DNA reference. Novel variants were further evaluated using laboratory developed bioinformatic tools.

**Results:** Sixty eight percent of patients with TMA had genetic variants in multiple genes as compared to 9% of patients without TMA, who had very few variants ( $p < 0.0001$ ). The median number of gene variants (known and unknown significance) seen in recipients with TMA was 1 (0-7), and 0 (0-2) in those without TMA ( $p < 0.0001$ ). No known pathogenic variants were seen in recipients without TMA. TMA incidence and the number of gene variants detected was much higher in non-Caucasian transplant recipients as compared to Caucasian recipients (3.1 vs 0.8,  $p < 0.0001$ ). Importantly,  $\geq 3$  gene variants were associated with very high transplant related mortality (83%) and were identified only in non-Caucasians with TMA. Overall survival in non-Caucasian recipients with TMA was inferior as compared to Caucasian recipients with TMA ( $p = 0.04$ ).

**Conclusion:** Our data indicate that multiple genetic variants that allow rapid activation of complement are present in non-Caucasian transplant recipients and likely predispose them to severe transplant-associated TMA resulting in high transplant related mortality, while this phenomenon is not observed in Caucasian transplant recipients. These findings may provide novel insights into racial disparities reported in transplant outcomes.

## FAMILIAL HAPLOIDENTICAL (FHI) T-CELL DEPLETED (TCD) WITH T-CELL ADBACK STEM CELL TRANSPLANTATION FOR PATIENTS WITH HIGH-RISK SICKLE CELL DISEASE (SCD) (IND 14359)

Julie-An Talano, Cori Abikoff, Carolyn Keever-Taylor, Mark C. Walters, Shalini Shenoy, Theodore Moore, Susan K. Parsons, Allen J. Dozor, Deborah Friedman, Qiuhu Shi, Suzanne

Braniecki, Brenda Grossman, Rona S. Weinberg, Erin Morris, Phyllis Brand, Sandra Foley, Olga Militano, Janet Ayello, Mildred Semidei-Pomales, Lee Ann Baxter-Lowe, and Mitchell S. Cairo

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**Background:** AlloSCT from HLA-matched MSD has been successful for high-risk SCD, and is the only known curative therapy (Talano/Cairo et al EJH, 2014). We have recently demonstrated 100% EFS and absence of sickle cell symptoms following reduced toxicity conditioning in HLA MSD or cord blood AlloSCT (Bhatia/Cairo et al BMT, 2014). However, the majority of children lack an HLA MSD, identifiable unrelated donors (URD) in this patient population are limited, and results from UCB are poor. We demonstrated CD34+ selection followed by T cell addback from URD in pediatric recipients with both malignant/nonmalignant disease led to 100% engraftment with minimal aGVHD (Geyer/Cairo et al BJH, 2011). FHI TCD AlloSCT could expand the donor pool and improve outcomes for patients with high risk SCD.

**Methods:** This SCD consortium trial (8 institutions) is investigating the safety, feasibility, EFS, donor chimerism, graft failure, aGVHD and cGVHD, and infectious mortality after FHI TCD AlloSCT in high-risk SCD patients (Figure 1). High risk included:  $\geq 1$  CVA,  $\geq 2$  ACS,  $\geq 3$  VOC in past 2 years, or 2 abnormal TCDs. Patients (2-<21 yrs) who have  $\geq 1$  high-risk SCD features were eligible. Patients received hydroxyurea and azathioprine, day -59 – day -11, fludarabine ( $150\text{mg}/\text{m}^2$ ), busulfan ( $12.8\text{mg}/\text{kg}$ ) thiotepa ( $10\text{ mg}/\text{kg}$ ), cyclophosphamide ( $200\text{mg}/\text{kg}$ ), R-ATG ( $8\text{mg}/\text{kg}$ ), and TLI ( $500\text{cGy}$ ) followed by FHI T-cell depleted AlloSCT. AGVHD prophylaxis: tacrolimus. We utilized the CliniMACS to enrich for peripheral blood HPC's; target dose of  $10 \times 10^6$  CD34+ cells/kg with  $2 \times 10^5$  CD3+ T cells/kg. Neurocognitive screening, to assess for associated cognitive issues, is completed.

**Results:** Eight patients have received AlloSCT to date. All patients utilized maternal donors who encountered no complications during collection. Evaluable pts had early neutrophil engraftment (median day +9),  $\geq 94\%$  whole blood chimerism and  $\geq 91\%$  RBC donor chimerism, no aGVHD or cGVHD. One patient developed late hepatic SOS and died at day +59; the remainder are alive and free of SCD symptoms (day +7 to +778).

**Conclusion:** Early results indicate FHI TCD AlloSCT is feasible in high-risk SCD patients who lack a MSD or URD. A larger cohort with longer term follow-up is needed to assess long-term safety and outcomes.

PBMTC Platform Session 5002

## **REDUCED INTENSITY CONDITIONING AND ALLOGENEIC STEM CELL TRANSPLANTATION (ALLOSCT) FOLLOWED BY TARGETED CONSOLIDATION IMMUNOTHERAPY WITH GEMTUZUMAB OZOGAMICIN (GO) IN CHILDREN AND ADOLESCENTS WITH CD33+ ACUTE MYELOID LEUKEMIA (AML)**

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**Background:** Myeloablative conditioning (MAC) AlloSCT in children with AML in CR1 is associated with 60% EFS but is associated with transplant-related mortality (TRM) and late effects. RIC-AlloSCT in children is safe and associated with less adverse effects. GO induces response in 30% of CD33+ pediatric relapsed AML.

**Objective:** To determine the safety and efficacy of RIC-AlloSCT followed by GO in children with CD33+ AML in CR1/CR2.

**Methods:** Conditioning: fludarabine 30mg/m<sup>2</sup> x 6 days, busulfan 3.2-4mg/kg x 2 days and alloSCT from matched related/unrelated donors. GO was administered  $\geq 55$  days post-alloSCT in 2 doses (8 weeks apart), following a dose escalation design.

**Results:** Thirteen patients, 10 CR1, 3 CR2, median age 14.5 years (range: 11-21); M:F:7:6. Eleven patients received alloSCT from 5-6/6 HLA-matched family donors: 8 PBSCs, 2 BM and 1 UCB. Two patients received unrelated grafts (one 4/6 and one 9/10 HLA-matched donor). Neutrophil and platelet engraftment occurred at median 14 days (range 7-31) and 18 days (range 10-52), respectively. Three patients received GO at dose level 1 (4.5 mg/m<sup>2</sup>/dose), 5 at dose level 2 (6 mg/m<sup>2</sup>/dose), 3 at dose level 3 (7.5 mg/m<sup>2</sup>/dose) and 2 at dose level 4 (9 mg/m<sup>2</sup>/dose). One patient experienced grade 3 transaminitis which resolved. Twelve patients experienced grade 4 myeloid toxicity; seven had grade 4 thrombocytopenia secondary to GO. Following the first dose of GO, neutrophil and platelet recovery were achieved at median 14 days (range 9-18) and 11 days (range 6-17), respectively. The second dose of GO was given at median 143 days (range: 120-209) post-alloSCT. Probability of grade II-IV aGVHD was 15.4%. Probability of OS following RIC-AlloSCT and GO consolidation at 1 and 3 years was 83.3% and 65.6%, respectively. No SOS was observed.

**Conclusion:** This preliminary data demonstrates that RIC followed by AlloSCT and consolidation with GO appears to be safe in children with CD33+ AML in CR1/CR2. A larger cohort with longer follow-up is required to determine long-term clinical significance.

**References:**

Del Toro/Cairo et al, BMT, 2004  
Satwani/Cairo et al, BBMT, 2013  
Sievers et al, JCO, 2001  
Woods et al, Blood, 2001

PBMTC Platform Session 5003

## **ROLE OF NATURAL KILLER CELLS IN CONTROLLING EWING SARCOMA GROWTH**

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**Background:** Ewing Sarcoma (ES) is the second most common sarcoma in childhood. Despite an overall improved outcome for localized ES with aggressive combination treatment, metastatic and relapsed ES remain as major challenges. For these patients, new therapy options are desperately needed. In recent years, cell- based immunotherapy has brought new hope to cancer patients. Natural Killer (NK) cells are immune cells with potential broad applications particularly in solid tumors. Specifically, ES cell lines were shown to be sensitive to NK cells killing in vitro. However, the utility of allo-NK cells against ES in vivo has not been fully explored. We can now expand NK cells ex vivo to overcome the cell number limitation in the clinical application. In addition, our data show that ex vivo Glycogen synthase kinase-3 (GSK-3) inhibition potentiates NK cells cytotoxicity.

**Objective:** To study whether ex vivo expanded human NK cells can control ES tumor growth in vivo and whether GSK-3 inhibition synergizes NK cytolytic activity against ES in vivo and in

vitro.

**Design/Method:** We inoculated 105 ES cell line, TC-106, subcutaneously into 4 groups of NOD-SCID-gamma (NSG) mice: 1) tumor only; 2) tumor with weekly injections of  $5 \times 10^7$  ex vivo K562-membrane-IL21 expanded NK cells i.v. starting on day 0; 3) same as in (2) with NK cells injected intra-tumorally; 4) same as in (2) with NK cells that were treated ex vivo with GSK-3 inhibitor for 4 hours prior to infusion. We assessed tumor growth and analyzed immune responses using a combination of molecular and cellular immunological tools and intravital two-photon imaging.

**Results:** Using artificial antigen-presenting cells, K562, transduced to express membrane-bound IL21, we were able to rapidly expand human NK cells by more than a thousand folds in 2 weeks. Our data also demonstrated enhanced NK-mediated cytotoxicity against human tumor cells in vitro after 4-hour exposure to GSK-3 inhibitor. Additional results from the in vivo experiments will be discussed at the conference.

**Conclusion:** Available data suggest that ES is sensitive to NK cells, whose function can be enhanced by GSK-3 inhibition. Our project suggests the feasibility of “off-the-shelf” NK-based cellular product for the treatment of ES.

PBMTC Platform Session 5004

## **DEFIBROTIDE FOR THE TREATMENT OF SEVERE HEPATIC VENO-OCCLUSIVE DISEASE: A SUBGROUP ANALYSIS OF CLINICAL BENEFIT FOR PEDIATRIC PATIENTS AS DETERMINED BY NUMBER NEEDED TO TREAT (NNT) TO ACHIEVE COMPLETE RESPONSE AND TO IMPROVE SURVIVAL AT DAY 100 POST HEMATOPOIETIC STEM CELL TRANSPLANT**

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**Background:** Hepatic veno-occlusive disease (VOD; also sinusoidal obstruction syndrome) is a serious complication of hematopoietic stem cell transplantation (HSCT). Severe VOD (sVOD) is associated with multi-organ failure; mortality is >80%. There are no FDA-approved treatments for sVOD. The data presented are based on efficacy analyses provided to the European Medicines Agency that formed the basis of the defibrotide approval in the European Union for the treatment of severe hepatic VOD following HSCT.

**Objective:** To calculate numbers needed to treat (NNTs) for defibrotide-treated pediatric patients to achieve one complete response (CR) and prevent one death at day+100 post-HSCT vs historical controls (HC) not receiving defibrotide and to evaluate how defibrotide compared to treatments for other acute life-threatening conditions.

**Design/Method:** A pivotal phase 3 study examined efficacy and safety of defibrotide 25 mg/kg/day in pediatric and adult patients with sVOD (44 pediatric patients aged  $\leq 16$  years) compared with HC (14 pediatric patients). The primary endpoint was CR (improvements in bilirubin and resolution of multi-organ failure [renal and/or pulmonary dysfunction]) by day+100 post-HSCT; secondary endpoints included survival at day+100. We calculated NNTs for defibrotide-treated pediatric patients to achieve one CR and to prevent one death at day+100 post-HSCT vs HC who did not receive defibrotide. NNT is the reciprocal of the absolute risk reduction ( $1/\text{ARR}$ ); ARR equals the control minus experimental event rates. To compare these



NNTs with NNTs in other studies, a literature search was conducted, identifying recent clinical trials in acute conditions with high short-term mortality.

**Results:** 36.4% of defibrotide-treated pediatric patients and 7.1% of the pediatric HC ( $P=0.0364$ ) achieved CR by day+100, for an NNT of 3 ( $1/(0.364-0.071)$ ). Day+100 survival was 50.0% for defibrotide pediatric patients and 35.7% for pediatric HC ( $P=0.351$ ), for an NNT of 7 ( $1/(0.500-0.357)$ ). NNTs from the literature ranged from 1–59.

**Conclusion:** This pivotal phase 3 trial showed clinically relevant improvement in CR and survival in a pediatric subgroup of defibrotide-treated patients vs HC not receiving defibrotide for sVOD. The NNTs to achieve these benefits proved comparable/lower than NNTs for other therapeutic medical interventions in critical care.

PBMTC Platform Session 5005

## IMPLEMENTATION OF NEWBORN SCREENING FOR SEVERE COMBINED IMMUNODEFICIENCY IN OREGON

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**Background:** Oregon implemented screening for severe combined immunodeficiency (SCID) in May 2014 by incorporating an assay for T-cell receptor excision circles (TRECs) into the newborn screen (NBS). Low or absent levels of TREC are suggestive of primary immunodeficiency. Twenty-four states have implemented NBS for SCID.

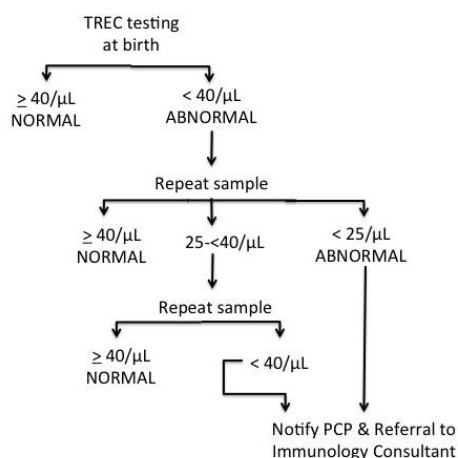
**Objective:** We report the outcomes of the SCID NBS program in the state of Oregon during the first six months of implementation.

**Design/Method:** From July–December 2014, TREC testing using a validated assay was obtained on all newborns undergoing NBS within 48 hours of birth. Infants with an abnormal test (TREC < 40/mcL blood) underwent repeat testing based on an established algorithm (see Figure 1). Notification to primary provider and referral to an immunology consultant were done for infants with abnormal testing.

**Results:** Of 33,251 infants screened, 85 had an abnormal TREC result on their first screen. Of these, four patients were found to have T-cell lymphopenia on follow-up testing. Two of these patients had confirmed SCID, one male with RAG2 mutation and one female with adenosine deaminase deficiency. The first infant underwent unrelated donor bone marrow transplant at age 3 months and is alive and well at 115 days post-transplant. The second patient is receiving enzyme replacement and is undergoing workup for gene therapy trial. Of the 2 patients with non-SCID lymphopenia, one was diagnosed with CHARGE syndrome and the other with DiGeorge syndrome.

**Conclusion:** During the first six months of screening, we identified two infants with SCID yielding an incidence of 1 in 17,000 live births, somewhat higher than that previously reported by other screening programs. NBS for SCID is a validated and effective intervention that has been successfully implemented in the state of Oregon. Our state lab serves as the laboratory for five other states, and plans are under way to implement testing in these locations.

Figure 1



PBMTC Platform Session 5006

## HISTOLOGIC FEATURES OF INTESTINAL THROMBOTIC MICROANGIOPATHY IN PATIENTS WITH HIGH RISK TMA AFTER HSCT

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**Objective:** We showed that high risk transplant-associated thrombotic microangiopathy (TMA) can present with multisystem involvement and has poor outcome after HSCT with <20% 1-year survival (Jodele, Blood 2014). TMA may involve intestinal vasculature and can present with bleeding and ischemic colitis. There are no established pathologic criteria for diagnosis of intestinal TMA (iTMA). The goal of our study was to review available tissue specimens obtained after HSCT in order to identify histologic features of iTMA.

**Design/Method:** Fifty consecutive HSCT patients who underwent endoscopy for gastrointestinal symptoms were evaluated for histopathologic signs of iTMA using 8 histologic criteria described in literature: mucosal hemorrhages, loss of glands, schistocytes, fibrinoid debris in the vessel lumen, intravascular microthrombi, endothelial cell swelling, endothelial cell separation and total denudation of mucosa. The reviewing pathologist was blinded to patients' clinical history. Histologic markers were listed as present or absent. For patients having multiple evaluations after HSCT, the first diagnostic tissue sample was used for this study. Patients were divided into 3 clinical groups based on the presence or absence of systemic TMA and intestinal graft versus host disease (iGVHD): TMA/iGVHD, no TMA/iGVHD, and noTMA/no iGVHD. Systemic TMA was diagnosed using rigorous clinical and diagnostic criteria. Comparison among the groups was done using Fisher exact test.

**Results:** Fifteen patients (30%) had a clinical diagnosis of systemic TMA. Out of 35 patients without TMA, 21 had clinical and histologic evidence of gut GVHD while 14 did not. Incidence of stage 3-4 gut GVHD was similar in the TMA/iGVHD and noTMA/iGVHD groups (79% vs 64%). All histologic signs of iTMA except for mucosal hemorrhages and endothelial cell swelling were significantly more common in patients with systemic TMA ( $p < 0.05$ ). Intravascular thrombi were exclusively seen only in patients with systemic TMA.

**Conclusion:** We identified histologic features of iTMA that can be used to delineate vascular injury of the bowel in patients with TMA after HSCT. Recognition of these histological signs in patients with gastrointestinal symptoms after HSCT may guide clinical decisions.

## **SURVIVAL OF PATIENTS WHO DEVELOP SOLID TUMORS FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANTATION**

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**Background:** Allogeneic hematopoietic cell transplantation (HCT) is a curative treatment for many benign and malignant conditions. Cure is often associated with late occurring adverse effects of therapy, including development of secondary solid cancers. While most reports address risk factors, outcomes for individuals who develop secondary solid cancers are incompletely described.

**Objectives:** The objective of this study was to estimate the probability of overall survival in transplant recipients dependent on secondary solid cancer subtype.

**Design/Method:** We evaluated outcomes in a previously identified cohort of patients who developed secondary solid cancers following allogeneic transplants registered to the Center for International Blood and Marrow Transplant Research. Patient, donor, and transplant characteristics were collected and descriptive statistics were generated. Probability of overall survival was calculated using the Kaplan-Meier estimator.

**Results:** There were 146 patients from our previously identified cohort who had developed secondary solid cancers and in whom we obtained follow up data. After exclusion of basal cell carcinomas (n=28) and those diagnosed at the time of autopsy (n=6), 112 individuals with secondary solid cancers were retained for survival analysis. Thirty patients were less than 20 years of age at the time of transplant. The median duration of follow-up from the time of secondary cancer development for survivors was 11.9 years (range: 0.8 – 23.4) and 75% of patients were followed for more than 7.0 years. The 5- and 10-year overall survival probabilities were 50% (95% CI: 41 – 60%) and 46% (95% CI: 37 – 57%), respectively. Overall survival was dependent upon secondary cancer type. Secondary cancer was the cause of death in the majority of patients who died following the development of melanoma, central nervous system, oral cavity, thyroid, lung, lower gastrointestinal tract, and bone secondary solid cancers.

**Conclusion:** Extended follow-up in this large cohort allowed for the most comprehensive longitudinal evaluation to date of an otherwise uncommon condition. These findings will enhance the ability of clinicians to predict outcomes for transplant survivors who develop secondary solid cancers.

## **TREATMENT OUTCOME OF SECONDARY AML AND MDS FOR CHILDREN AND YOUNG ADULTS FOLLOWING MYELOABLATIVE HEMATOPOIETIC STEM CELL TRANSPLANTATION**

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**Background:** Allogeneic hematopoietic stem cell transplantation (HSCT) is considered standard of care in the treatment of therapy related MDS/AML (t-MDS/t-AML).

**Objectives:** We sought to update outcomes of children and young adults (CAYA) with t-MDS/t-AML undergoing HSCT.

**Design/Method:** A retrospective analysis of CAYA patients' age <26 years with t-MDS/ t-AML treated with HSCT at University of Texas MD Anderson Cancer Center was conducted. The outcome of 32 patients with t-MDS /t-AML following myeloablative chemotherapy and HSCT is reported using descriptive measures.

**Results:** Thirty-two patients (median age 20, range 2 to 25) with t-MDS (n=8) and t-AML (n=24) underwent allogeneic HSCT between 1990 and 2013; twenty-four patients were male. Primary diagnosis: severe aplastic anemia (n=6), acute lymphoblastic leukemia (n=5), osteosarcoma (n=5), Hodgkin's lymphoma (n=4), germ-cell tumor (n=4), non-hodgkin lymphoma (n=4), and others (n=3). Underlying malignancies were treated with chemotherapy in 17 patients, combined chemotherapy and radiation in 6 patients, immune-modulatory agents in 3 patients, and autologous HSCT in 2 patients (6%). 24/32 patients received salvage chemotherapy prior to transplant. Among them, 12/24 (50%) patients were transplanted with persistent morphological disease. Donor sources include; marrow (n=15), mobilized apheresis (n=9), and umbilical cord blood (n=8). All patients received myeloablative conditioning; mainly busulfan based. 17 patients (53%) had matched unrelated graft and 3 patients had haploidentical transplant. Twenty-eight (87%) recipients achieved successful donor engraftment. Acute graft versus host disease (aGVHD) occurred in 19 patients; 3 (15%) with grade 3 to 4. At a median follow-up of 5.2 years (Range: 0.04-20.95), 15 patients (47%) relapsed, including 8 patients were refractory to cytoreductive chemotherapy prior to transplant. 18 patients (56%) died during follow up. Cause of death: Disease recurrence (n=12), GVHD (n=2), Graft Failure (n=2), Infection and multi-organ failure (n=3).

**Conclusion:** This is the largest reported CAYA study of HSCT for t-MDS/t-AML. In this age group, myeloablative regimen was well tolerated with low rates of severe GVHD and transplant related mortality. Major cause of death was attributed to disease relapse/recurrence. Primary complete remission prior to transplant is an important predictor of post-transplant disease free survival.

PBMTC Platform Session 5009

### **PROSPECTIVE ECHOCARDIOGRAPHIC SCREENING FOR CARDIAC DYSFUNCTION 100 DAYS AFTER STEM CELL TRANSPLANT IN CHILDREN AND YOUNG ADULTS**

Christopher Dandoy, Thomas Ryan, John Jeffries, Michelle Cash, Ranjit Chima, Javier El-Bietar, Russel Hirsch, Adam Lane, Kasiani Myers, Zachary Paff, Stella Davies, Sonata Jodele

*Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, United States*

**Background:** Cardiac evaluation during first 100 days after stem cell transplant (SCT) is usually performed only if clinically indicated, however, patients with mild left ventricular (LV) dysfunction can be asymptomatic. Scheduled post-SCT screening at day 100 may identify patients with subacute cardiac toxicity identified as LV dysfunction.

**Design/Method:** We conducted a single center prospective study to screen for LV dysfunction after SCT in 100 consecutive patients. Patients received echocardiography screening prior to SCT and 100 days post-SCT. Patients were classified as having LV dysfunction if

echocardiography met at least one of the following criteria: (1) ejection fraction (EF) 50% or less, (2) fractional shortening (FS) two standard deviations below the age adjusted mean, (3) >10% decrease in EF or FS at day 100 from baseline.

**Results:** Ninety-two of 100 consecutive patients completed day 100 screening (8 died). 25% (23 of 92) had LV dysfunction at day 100 (Table). 96% (22 of 23) were asymptomatic at time of screening. Patients with LV dysfunction were older at the time of SCT than those without ( $p=0.068$ ). Patients receiving PBSC grafts had an increased incidence of LV dysfunction ( $p=0.032$ ), likely related to the diagnosis of those patients (malignancy receiving autologous SCT and Fanconi Anemia). Overall, however, diagnosis was not significantly associated with LV dysfunction. Patients with previous anthracycline exposure had increased LV dysfunction compared to those without (48% vs. 26%). GVHD, engraftment syndrome, and viremias in the first 100 days were not significantly associated with decreased RV function. Death at 1 year was similar in both groups.

**Conclusion:** Twenty five percent of patients showed signs of cardiac dysfunction at day 100, which was higher than anticipated. Although there were no acute differences in outcome, the long-term complications are unknown. Echocardiography at day 100 identifies SCT cardiac dysfunction; however, more research is needed to understand the overall impact on long-term outcome.

PBMTC Poster 5010

## **PEDIATRIC AND ADULT SUBGROUP RESULTS FROM AN ONGOING DEFIBROTIDE EXPANDED ACCESS PROGRAM IN THE US FOR PATIENTS WITH HEPATIC VENO-OCCLUSIVE DISEASE**

Stephan A. Grupp, Paul G. Richardson, Angela R. Smith, Brandon M. Triplett, Nancy A. Kernan, Joseph H. Antin, Leslie Lehmann, Maja Miloslavsky, Robin Hume, Alison L. Hannah, Bijan Nejadnik, Robert J. Soiffer

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**Background:** Hepatic veno-occlusive disease (VOD), or sinusoidal obstruction syndrome, is a potentially fatal complication of hematopoietic stem cell transplantation (HSCT). Severe VOD (sVOD) has been associated with >80% mortality; it is characterized clinically by multi-organ failure (MOF). In the EU, defibrotide is approved for treatment of severe hepatic VOD in HSCT. In the US, defibrotide is available through an expanded-access, protocol-directed treatment IND (T-IND) in children and adults with VOD following HSCT/chemotherapy.

**Objectives:** Analyze day+100 survival post-HSCT/chemotherapy for pediatric/adult subgroups in this ongoing defibrotide VOD study.

**Design/Method:** Patients with VOD received defibrotide 6.25 mg/kg q6h, 2h infusion, for a recommended  $\geq 21$  days. sVOD was diagnosed by Baltimore criteria (bilirubin  $\geq 2.0$  mg/dL with  $\geq 2$  of hepatomegaly, ascites, or 5% weight gain) plus MOF (renal and/or pulmonary); non-sVOD (ie, without MOF) was diagnosed per modified Seattle criteria ( $\geq 2$  of ascites, bilirubin  $\geq 2.0$  mg/dL, hepatomegaly/upper right quadrant pain, or—in this study—5% weight gain).

**Results:** Among patients enrolled from December 2007-December 2013, 641 received  $\geq 1$  dose. Of 636 patients with available age, 58% were pediatric (aged  $\leq 16$ y), and 42% were adult ( $>16$ y). Among pediatric patients, 28% were aged 0-23 months, 52% were 2-11y, and 20% were 12-16y. Among post-HSCT pediatric and adult patients, respectively, day+100 survival was 58% (163/283) and 45% (109/243). sVOD occurred in  $\geq 50\%$  of both subgroups. Among sVOD and

non-sVOD subgroups, respectively, pediatric survival was 50% (79/157) and 67% (84/126); adult survival was 38% (46/122) and 52% (63/121). For post-chemotherapy pediatric and adult patients, respectively, survival rates were 83% (39/47) and 60% (9/15); sVOD subgroup rates were 77% (20/26) and 67% (4/6). Adverse events (AEs) occurred in 61% of (227/372) children and 76% of (200/264) adults, with treatment-related AEs in 19% and 24%, respectively. Serious AEs occurred in 45% of pediatric patients (most common non-VOD/non-MOF: pulmonary hemorrhage [8%]) and 53% of adults (most common non-VOD/non-MOF: hypotension [5%]). **Conclusion:** Defibrotide showed favorable day+100 survival rates and was generally well-tolerated, with manageable toxicity. Survival was especially promising among pediatric and adult subgroups with non-sVOD, which may be a consideration for earlier treatment before sVOD develops. T-IND enrollment continues.

PBMTC Poster 5011

## HSCT OUTCOMES OF NATIVE AMERICANS WITH C-MPL MUTATIONS

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**Background:** Congenital amegakaryocytic thrombocytopenia (CAMT) is one of a group of rare autosomal recessive bone marrow failure syndromes. Patients usually present with isolated thrombocytopenia and reduction or absence of megakaryocytes in the bone marrow without other physical anomalies. Patients may present with pancytopenia or progress toward bone marrow failure. Mutations in the Thrombopoietin (TPO) receptor gene c-MPL {1} cause this disease. The only curative treatment for CAMT is hematopoietic stem cell transplant (HSCT).

Patient ID	Gender	Race/Ethnicity	Platelet Count	c-MPL mutations	Donor Type/Match	Conditioning	Transplant Date	Day 100 survival(last f/u)	GVHD
Pt. 1	F	Native American/Choctaw	14K, No anomalies	c.268 C>T, (p.Arg90Stop) c.1609 C>T, p.Arg537Trp	Sibling Marrow Donor, 5/6	Atgam, Cytoxan	3/11/1998	Yes (7/13/14)	Acute, resolved
Pt. 2	F	Native American/Choctaw	26K, No anomalies	c.268 C>T (p.Arg90Stop) c.1609 C>T (p.Arg537Trp)	Unrelated Cord, 5/6	Busulfan, Cytoxan	5/8/2009	Yes (4/3/14)	Acute resolved
Pt. 3	M	Native American/Choctaw	14K, No anomalies	c.268 C>T, (p.Arg90Stop) c.1609 C>T, p.Arg537Trp	Sibling Marrow Donor, 6/6	Busulfan, Cytoxan	8/19/2010	Yes (1/27/14)	None
Pt. 4	M	Native American/Choctaw	24k, No Anomalies	c.268 C>T (p.Arg90Stop) c.1609 C>T (p.Arg537Trp)	Unrelated Cord, 5/6 Cord, 5/6	Busulfan, Atgam, Cytoxan	6/10/2011 7/26/2011	Yes (deceased)	None
Pt. 5	F	Native American/Choctaw	9K, No Anomalies	Homozygous c.1609 C>T (p.Arg537Trp)	Unrelated Cord, 5/6	Busulfan, Atgam, Cytoxan	3/22/2013	Yes (12/15/14)	Acute resolved

**Conclusion:** Mississippi has a large population of Native Americans, Choctaws who live in reservation communities. All of the patients presented with <40K platelets and without other anomalies. Patients 1-4 demonstrate the same c-MPL mutations. Patient 5, half-sibling of

patient 1, is homozygous for p.Arg537Trp. Patients 1 and 3 had matched sibling donors with successful engraftment. Patients 2 & 5 were transplanted using unrelated 5/6 cords. Patient 4 failed engraftment of double 5/6 cord and a second single 5/6 cord and later succumb to fungal sepsis five months after HSCT. Long term morbidity and mortality has been without significant GVHD. Genetic counseling was offered to all families since transplant is known to cure the disease in most patients even when mismatched donor sources are the best option available.

**References:** Ihara K, Ishii E, Eguchi M, et al. Identifications of mutations in the c-mpl gene in congenital amegakaryocytic thrombocytopenia. Proc Natl Acad Sci USA 1999

PBMTC Poster 5012

## **DAY+100 SURVIVAL ANALYSIS BY PRIOR HEMATOPOIETIC STEM CELL TRANSPLANT TYPE FROM AN ONGOING US STUDY OF DEFIBROTIDE FOR HEPATIC VENO-OCCLUSIVE DISEASE**

Stephan A. Grupp; Paul G. Richardson; Angela R. Smith; Brandon M. Triplett; Nancy A. Kernan; Joseph H. Antin; Leslie Lehmann; Maja Miloslavsky; Robin Hume; Alison L. Hannah; Bijan Nejadnik; Robert J. Soiffer

*The Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, United States*

**Background:** Hepatic veno-occlusive disease (VOD, also known as sinusoidal obstruction syndrome) is a potentially fatal complication of hematopoietic stem cell transplant (HSCT). Severe VOD (sVOD), which has been associated with a mortality rate >80%, is clinically defined as VOD with multi-organ failure (MOF). In the European Union, defibrotide is approved for treatment of severe hepatic VOD in HSCT. In the US, defibrotide is available through an expanded-access; protocol-directed treatment IND (T-IND) collecting safety/efficacy data in VOD post HSCT/chemotherapy.

**Objectives:** To analyze day+100 survival post-HSCT in autologous and allogeneic subgroups from the ongoing US T-IND study of defibrotide in VOD.

**Design/Method:** Defibrotide was administered to VOD patients in 2-hour infusions, 6.25 mg/kg q6h (25 mg/kg/d), for  $\geq 21$  days. Selection criteria: diagnosis of VOD per Baltimore or modified Seattle criteria post HSCT/chemotherapy, with or without MOF. Exclusion criteria: clinically significant bleeding or need for  $\geq 2$  vasopressors. Assessments include safety and day +100 survival.

**Results:** 641 patients (enrolled December 2007–December 2013), including post-chemotherapy patients without HSCT, received  $\geq 1$  defibrotide dose. 57% of patients were male, median age was 13y (range 0-69) with 58% (372/636)  $\leq 16$ y. Most common underlying diagnoses included acute myelogenous leukemia (27%) and acute lymphocytic leukemia (23%), and most common graft vs host disease agents were tacrolimus (41%), cyclosporine (30%), and methotrexate (29%). Of 336 patients with sVOD and HSCT, 54% were male, median age was 12y (range 0-69) with 61% (204/333)  $\leq 16$ y. For 526 post-HSCT patients with available survival data, most were allografts (n=467) vs autografts (n=56); graft type not available n=3. Among allografts and autografts, 54% and 48%, respectively, had sVOD. Day+100 survival for allografts was 50% (95% CI 46–55) overall, 43% (37–49) for sVOD, and 58% (52–65) for non-sVOD. For autografts it was 66% (54–79) overall, 59% (41–78) for sVOD, and 72% (56–89) for non-sVOD.



**Conclusion:** In this trial, day+100 survival rates in both allograft and autograft sVOD subsets were consistent with prior defibrotide studies. The higher survival rates in the non-sVOD subsets indicate further study is warranted to determine impact of treatment earlier in the course of VOD.

PBMTC POSTER 5013

### **EVALUATION AND ANALYSIS OF CAUSES OF EARLY READMISSION FOLLOWING UMBILICAL CORD BLOOD TRANSPLANTATION IN CHILDREN, ADOLESCENTS AND YOUNG ADULTS**

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**Background:** Umbilical cord blood (UCB) serves as a source of allogeneic hematopoietic stem cells for patients lacking a suitable donor. Delayed immune reconstitution after UCB transplantation (UCBT) poses an increased risk for opportunistic infections, and relapse. Patients presenting with opportunistic infections early after UCBT has been shown to be a predictor of overall and event-free survival (OS/EFS) in these recipients.

**Design/Method:** A retrospective analysis of patients less than 26 years old who were readmitted after UCBT at MD Anderson Cancer Center was conducted using descriptive measures.

**Results:** 371 consecutive transplants in children and young adult (CAYA) age group from 2008 to 2013 revealed a total of 56 UCB transplants. The median age at time of UCBT was 16.8 years. Hematologic malignancy was the main indication for transplantation (94%), with 55% of patients having positive minimal residual disease. Lansky/Karnofsky performance status was at median of 90%. Twenty-four (71%) patients underwent double UCBT. One third of the patients presented acute graft versus host disease. 34 (60%) UCBT patients had their first readmission within 6 months following transplant and the median number of readmissions per patient was two. A total of 68 readmission events were reported in 34 patients. The primary etiology of these readmissions was due to documented infections accounting for 46% of the events. Of those, 22% due to bacteria whereas 56% were due to viruses as follows, BK virus (n=9), RSV (n=4) accounted for 65% of viral infections, followed by parainfluenza and cytomegalovirus reactivation/infection with 24%, finally EBV and rotavirus with 11%. Relapse accounted for 15% (n=5) of the first readmissions. At a median follow up of 23.5 months (range 0.2 to 72), 15 of 33 readmitted patients remain alive. Causes of death were due to disease recurrence (n=14) and transplant related events (n= 5), mostly infection associated.

**Conclusion:** This CAYA cohort reports the etiology of readmission within 6 months after UCBT. Viral Infection was the highest risk factor for readmission. Disease relapse contributes to the highest mortality. These data highlight complications of delayed immune reconstitution and the need to augment immunity for viral infections on these immunocompromised recipients.

PBMTC Poster 5014

### **EARLY INTEGRATION OF PALLIATIVE CARE FOR CHILDREN AND ADOLESCENTS UNDERGOING HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)**



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**Background:** HSCT is a treatment modality with significant symptom distress. Multiple pediatric professional organizations set a standard to enhance quality of life.

**Objectives:** To investigate the feasibility and impact of early integration of palliative care services (PCS) to promote comfort in children undergoing HSCT.

**Design/Method:** Building on an initial single-site feasibility study, we examined the primary aims of: willingness of patients/families to receive PCS; willingness of healthcare team to refer families; resource allocation; and family/provider satisfaction. Interventions, beyond HSCT standard care, included supportive care counseling and other integrative therapies. Data collection included referral and enrollment patterns, resource allocation, family and provider satisfaction, and child self-report and parental reports of their child's comfort using validated scales.

**Results:** 100% of eligible families were referred and consented to participate (N = 41 for 454 encounters). Level of comfort was maintained throughout the trajectory (baseline mean (M) = 3.6/4, 14-21 days post-HSCT M = 3.22/4 and discharge M = 4/4,  $z = -.816$ ,  $p = .414$ ). There was no difference in comfort between diagnostic groups,  $X^2(2) = .080$ ,  $p = .961$  or for type of HSCT,  $X^2(2) = .939$ ,  $p = .625$ . Family satisfaction was high indicating comfort in receiving palliative care (M = 4.9/5), very helpful in managing symptoms and stresses (M = 4.8/5), improved access to services (M = 4.6/5). Families felt it was very important to offer PCS and that they were very likely to recommend these services to others (M = 5/5), and that they were more likely to recommend the institution as a transplant center to other families based upon their palliative care experiences (M = 4.7/5). Provider satisfaction was overall high (M = 4.4/5). Providers also indicated that it was very important that PCS be offered (M = 4.8/5) and they were very likely to recommend services to patients and families (M = 4.6/5).

**Conclusion:** Early palliative care consultation is now a standard of care for HSCT admissions and a novel approach as supportive care concurrent with curative intent therapy. Comfort was maintained rather than significantly declining as is usually anticipated with intensive HSCT therapies.

PBMTC Poster 5015

## **FACTORS ASSOCIATED WITH IMPROVED OUTCOMES AFTER SECOND HEMATOPOIETIC CELL TRANSPLANTATION IN PATIENTS WITH LEUKEMIA**

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**Background:** Recurrent leukemia after hematopoietic cell transplantation (HCT) is associated with poor prognosis, and is the most frequent indication for a second transplant. While there have been published reports of second HCT transplants, this is one of the largest single institution studies in terms of number of total transplants, number of pediatric patients and number of second grafts using cord blood.

**Objectives:** We sought to analyze the factors associated with increased survival of leukemic patients who required a second HCT after disease relapse.

**Design/Method:** A retrospective review of 71 patients aged 1-65 years who underwent a second HCT at the University of Arizona (Tucson, AZ, USA) between 1992 and 2011 was performed.

**Results:** Overall, 42.2%, and 32.4% of patients were alive and disease free at one and five years respectively after the second SCT. Risks of death were significantly higher in patients who received their second transplant < 1yr versus > 1yr) was also significant when each type of leukemia (ALL n= 32, p<0.004, AML n=25, p<0.0003 and CML n=12, p<0.0002) was analyzed separately. Myeloablative conditioning and receiving TBI for the second transplant resulted in increased 5-yr survival (42.5 vs. 19.4% p<0.04 and 44.8 vs. 23.5% p<0.05 respectively). Age also predicted better outcomes with patients under 21 years (n=49) having a 40.8% survival at 5 years compared to 0% in patients older than 50 (p<0.006). The sources of second grafts were from matched siblings (n=37), unrelated cord blood (n=18), unrelated donors (n=14) and autologous (n=2). Potential advantage of using a different donor for the second transplant was not a significant factor, in our study nor were remission status, diagnosis, source of stem cells and the era that transplants were performed.

**Conclusion:** In this large single institution study we confirmed that a second transplant remains a viable curative option for patients that relapse after first HCT. Duration between transplants, younger age, myeloablation and/or TBI for the second transplant appear to be the most important determinants of improved outcome.

PBMTC Poster 5016

## **PROSPECTIVE ECHOCARDIOGRAPHIC SCREENING FOR CARDIAC DYSFUNCTION 100 DAYS AFTER STEM CELL TRANSPLANT IN CHILDREN AND YOUNG ADULTS**

Christopher Dandoy, Thomas Ryan, John Jefferies, Michelle Cash, Ranjit Chima, Javier El-Bietar, Russel Hirsch, Adam Lane, Kasiani Myers, Zachary Paff, Stella Davies, Sonata Jodele

*Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, United States*

**Background:** Cardiac evaluation during first 100 days after stem cell transplant (SCT) is usually performed only if clinically indicated, however, patients with mild left ventricular (LV) dysfunction can be asymptomatic. Scheduled post-SCT screening at day 100 may identify patients with subacute cardiac toxicity identified as LV dysfunction.

**Design/Methods:** We conducted a single center prospective study to screen for LV dysfunction after SCT in 100 consecutive patients. Patients received echocardiography screening prior to SCT and 100 days post-SCT. Patients were classified as having LV dysfunction if echocardiography met at least one of the following criteria: (1) ejection fraction (EF) 50% or less, (2) fractional shortening (FS) two standard deviations below the age adjusted mean, (3) >10% decrease in EF or FS at day 100 from baseline.

**Results:** Ninety-two of 100 consecutive patients completed day 100 screening (8 died). 25% (23 of 92) had LV dysfunction at day 100 (Table). 96% (22 of 23) were asymptomatic at time of screening. Patients with LV dysfunction were older at the time of SCT than those without (p=0.068). Patients receiving PBSC grafts had an increased incidence of LV dysfunction (p=0.032), likely related to the diagnosis of those patients (malignancy receiving autologous SCT and Fanconi Anemia). Overall, however, diagnosis was not significantly associated with LV dysfunction. Patients with previous anthracycline exposure had increased LV dysfunction compared to those without (48% vs. 26%). GVHD, engraftment syndrome, and viremia in the first 100 days were not significantly associated with decreased RV function. Death at 1 year was similar in both groups.

**Conclusion:** 25% of patients showed signs of cardiac dysfunction at day 100, which was higher than anticipated. Although there were no acute differences in outcome, the long-term complications are unknown. Echocardiography at day 100 identifies SCT cardiac dysfunction; however, more research is needed to understand the overall impact on long-term outcome.

PBMTC Poster 5017

## **LATE ENDOCRINE EFFECTS REMAIN PREVALENT DESPITE REDUCED INTENSITY CHEMOTHERAPY FOR HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDREN AND YOUNG ADULTS**

Kasiani Myers, Jonathan Howell, Gregory Wallace, Christopher Dandoy, Javier El-Bietar, Adam Lane, Susan Rose, Stella Davies, Sonata Jodele

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**Background:** Myeloablative conditioning regimens used for hematopoietic stem cell transplant (HSCT) affect endocrine function, most commonly causing primary hypothyroidism and hypogonadism. Little is known regarding these late effects after reduced intensity conditioning (RIC) regimens without irradiation.

**Objective/Methods:** To evaluate late endocrine effects after RIC HSCT, an IRB approved retrospective chart review was performed of 120 children and young adults who received a single RIC HSCT without radiation between 2004 and 2012 and survived at least 1 year. Analysis was grouped by age (<2 years and  $\geq 2$  years), and diagnosis (HLH/XLP, other immune disorders (PID), and metabolic or genetic disorders).

**Results:** Subjects age 2-17y with height (n=103) and weight (n=120) data prior to and at least 1 year following HSCT were analysed for growth. Mean follow-up was 3.2 years. All groups displayed short stature before (height for age z-score (HAZ)= -1.33) and after HSCT (HAZ= -1.35) (p=0.66). After HSCT, younger children with HLH/XLP grew better (HAZ=-3.36 vs -1.35, p=0.002), while older subjects had worsening (HAZ=-0.61 vs -0.99, p=0.004), although all remained short. Overall, subjects receiving additional steroid therapy were shorter than untreated patients (p= 0.02). After HSCT, older subjects with HLH/XLP became thinner (BMI z-scores (BMI-Z)= 1.29 vs. 0.61, p=0.003), and similarly in metabolic or genetic disorders (BMI-Z= 0.56 vs. -0.77, p<0.001). There was a trend toward increased BMI-Z among younger children in these same groups. Thyroid function testing was performed on 77 subjects following HSCT. Eleven (14%) had evidence of primary hypothyroidism, 5 (7%) central hypothyroidism, and 2 (3%) had evidence of primary hyperthyroidism. Of the 66 subjects with 25-OH vitamin D levels, 46 (70%) were low (<30 ng/mL). Bone densitometry by DXA scan was below -1 SD in 16 of 21 evaluable subjects with an average Z-score of -1.8 SD (0.7 to -4.9 SD) at median duration after HSCT of 2.2 years.

**Conclusions:** Despite using RIC, children and young adults still have significant late endocrine effects following transplant. Algorithms for early detection should be implemented for RIC transplant survivors. Further research is required in order to compare post-transplant endocrine effects after RIC to those after standard chemotherapy protocols.

PBMTC Poster 5018

# **ROR1 EXPRESSING NEUROBLASTOMA (NB), MEDULLOBLASTOMA (MB), AND EWING'S SARCOMA (ES) CAN BE EFFECTIVELY TARGETED WITH NK CELLS MODIFIED TO EXPRESS AN ANTI-ROR1 CHIMERIC ANTIGEN RECEPTOR (CAR)**

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**Background:** Metastatic pediatric neuroectodermal solid tumors especially NB, MB, and ES have a dismal prognosis.<sup>1</sup> Targeted cellular therapy with T or NK cells modified with CARs is a novel approach to chemo-resistant tumors.<sup>2,3</sup> NK cells can be significantly expanded by co-culture with genetically engineered K562 cells overexpressing mb-IL21.<sup>4</sup> ROR1 has been identified as a novel target on B cell tumors in which CARs can be developed and utilized for targeted cellular therapy.<sup>5</sup>

**Objectives:** To evaluate the invitro cytotoxic activity and function of ex-vivo expanded PBNK (ExPBNK) with K562 mb-IL21 and nucleofected with mRNA encoding an anti-ROR1-CAR against NB, MB, and ES

**Design/Method:** PBNK were expanded with irradiated K562 Clone 9.mb-IL21. ExPBNK cells were electroporated with anti-ROR1-CD28-41BB1-CD3 $\zeta$ -tEGFR-mRNA. Cytotoxicity of ROR1 CAR-NK cells was investigated against NB (SKNBE2, SKNFI & SHSY5Y), MB (DAOY) and ES (TC71 and A673) cell lines by DELFIA assay. Intracellular staining of CD107a, interferon gamma, perforin and granzymeB was performed against tumor targets and analyzed on the MACSQuant flow cytometer

**Results:** NB, MB, and ES cell lines expressed ROR1 (50.2 $\pm$ 15.6%, 55.5 $\pm$ 5.1%, 31.5 $\pm$ 12%), respectively. Expansion of NK cells was significantly increased 3988 $\pm$ 435 fold (p=0.00001) at day 14 vs day 0. Nucleofection success was measured by F(ab')<sub>2</sub> expression and showed a significant increase in anti-ROR1-CAR- (88.3 $\pm$ 1.7%) vs Mock-electroporated NK cell populations (8.1 $\pm$ 6.9%) p=0.0001, at 36-48 hours. Anti-ROR1-CAR-NK significantly increased cell lysis compared to Mock NK (93 $\pm$ 4.6% vs 63.6 $\pm$ 7.4%) p=0.00001, against ROR1 expressing cell lines at 10:1 ET ratio. Similarly, expression of CD107a (46.1 $\pm$ 9.1 vs 27.6 $\pm$ 2.4%) p=0.001, Interferon Gamma (34.1 $\pm$ 11.6 vs 16.7 $\pm$ 6.7%) p=0.003, GranzymeB (68.5 $\pm$ 8.9 vs 46 $\pm$ 7.2%) p=0.002, and Perforin (51.3 $\pm$ 7.7 vs 30.3 $\pm$ 11.9%) p=0.002, were significantly increased in anti-ROR1-CAR-NK vs Mock-NK cells at 10:1 E:T ratio against the ROR1 expressing targets.

**Conclusion:** Anti-ROR1-CAR-ex-PBNK cells had significantly enhanced cytotoxicity and significantly increased CD107a, interferon gamma, perforin, and granzyme B activity against ROR1 expressing tumors. Future directions include investigating the ex-PBNK anti ROR1-CAR cells in-vivo against ROR1 expressing solid tumors.

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# VORICONAZOLE LEVELS INDICATE THAT PROPHYLAXIS DOSING MAY BE INSUFFICIENT FOR PROTECTION FROM FUNGUS IN PEDIATRIC STEM CELL RECIPIENTS

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**Background:** Voriconazole is routinely used for prophylaxis and treatment of invasive fungal infections in pediatric hematopoietic stem cell recipients (HSCT). Although therapeutic drug monitoring is recommended, there is no consensus about voriconazole dosing in pediatrics.

**Objectives:** To examine the relationship between voriconazole dosing, age, ethnicity, and therapeutic levels.

**Design/Method:** Retrospective chart review of 69 consecutive patients treated at our institution who received voriconazole following HSCT between 2009 and 2013 to determine if dose, age, and ethnicity influenced time to achieve therapeutic levels ( $\geq 1\mu\text{g/ml}$ ).

**Results:** Patients received allogeneic (n=64) or autologous (n=5) HSCT, mean age 10.19 years (7.6-12.7). The mean number of days to achieve therapeutic levels for all patients was 31 (95% CI 25-37.12). 37% of the patients had an undetectable level at first monitoring. Patients starting at doses higher than 4mg/kg achieved therapeutic levels significantly faster than those starting at 4mg/kg (p=0.0089). There were also significant differences between: (i) dose and days to achieve therapeutic levels, (ii) proportion of patients with an undetectable first level versus those taking longer than the cohort mean (Table) and (iii) the mean time to achieve therapeutic levels and age (p=0.03). There was insufficient power to examine ethnicity (p=0.63).

**Conclusion:** Voriconazole levels should be monitored even during prophylaxis. The lower recommended dosing does not achieve therapeutic levels until a mean of 31 days, rendering patients unprotected for fungal infection during the most vulnerable period. Therefore, to improve efficacy we recommend initiating voriconazole at higher doses to decrease the time to achieve optimal drug levels.

**Table:**

A. All patients (n=69)	Mean (95% CI)				
Days to therapeutic level ( $1\mu\text{g/ml}$ )	31 days (25-37.12)				
B. Starting dose	4mg/kg (n=42)	>4mg/kg (n=27)		P value	
First level ( $\mu\text{g/ml}$ )	0.54 (0.28-0.79)	2.6 (1.32-3.87)		0.002	
First therapeutic level ( $>1\mu\text{g/ml}$ )	1.83 (1.50-2.16)	3.5 (2.3-4.71)		0.0016	
Days to therapeutic level ( $1\mu\text{g/ml}$ )	37.5 days (29-46)	21.94 days (13.8-28.4)		0.0089	
Proportion of patients with first level undetectable	40% (17/42)	26% (7/27)		0.3	
Proportion of patients > than cohort mean days for therapeutic level	23/42 (54%)	5/22 (22%)		P=0.0003	
Proportion of patients taking > than cohort mean days for therapeutic levels based on ethnicity	Caucasians 29% (15/27)	African American 50% (15/30)	Asians 33% (2/6)	Hispanic 28% (2/7)	Others 33% (3/9)

## **DONOR CELL ENGRAFTMENT EVALUATION IN CEREBROSPINAL FLUID BY SHORT TANDEM REPEAT ANALYSIS FOLLOWING HEMATOPOIETIC STEM CELL TRANSPLANTATION**

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**Background:** The role of allogeneic hematopoietic stem cell transplantation (HSCT) has been debated in central nervous system leukemia and lymphoma. Graft-versus-leukemia/lymphoma effect mediated by donor lymphocytes is believed to be the main operational mechanism of a successful allogeneic HSCT. Sustained full donor chimerism in the bone marrow or peripheral blood is associated with successful engraftment and survival.

**Objectives:** To determine if short tandem repeat (STR) analysis could be used to determine donor chimerism status in the cerebrospinal fluid (CSF) cells following allogeneic HSCT.

**Design/Method:** Retrospective review of medical records was conducted in leukemia or lymphoma patients whose CSF samples were submitted following allogeneic HSCT for STR analysis due to an either underlying CNS disease or potential risk for CNS involvement. Ten different loci were studied and the average donor percentage of the informative loci was reported.

**Results:** Ten allogeneic HSCT recipients, seven with acute lymphoblastic leukemia (ALL), one acute myeloid leukemia, one Burkitt lymphoma and one blastic plasmacytoid dendritic cell neoplasm were included in the analysis. Six of the children had central nervous system disease at diagnosis and four were at high risk of involvement. All children received myeloablative conditioning. A total of 25 CSF samples were analyzed. Fifteen (60%) of the samples gave conclusive results. Bone marrow donor chimerism was 99% (91-100) and 100% in lymphoid and myeloid fractions at day +30 evaluations, respectively. Among the seven samples with conclusive results in initial sampling, median post-HSCT day of analysis was 97 (30-147); CSF cell donor chimerism ranged between 92-100%. Corresponding bone marrow samples revealed 99% (95-100) and 100% donor chimerism in lymphoid and myeloid cells, respectively. Among all the samples with conclusive results, mean CSF cell count was  $2.3/\text{mm}^3$  (0-8) and 86.5% (50-100) were lymphocytes. In one ALL case, CSF cells continued to show 100% donor chimerism at the time of systemic relapse.

**Conclusion:** These observations suggest that STR analysis following allogeneic HSCT could be used to monitor CSF cell donor chimerism status with an acceptable yield despite small number of cells available in samples.

## **LOW INCIDENCE OF HEPATIC VENO OCCLUSIVE DISEASE (VOD) USING URSODEOXYCHOLIC ACID (URSODIOL) AND HEPARIN PROPHYLAXIS IN PEDIATRIC HEMATOPOIETIC PROGENITOR CELL TRANSPLANT (HPCT) PATIENTS AT THE MEDICAL COLLEGE OF WISCONSIN**

A Vinitsky, JE Southwood, R Brazauskas, M Thakar, J Casper, D Margolis, J Talano

**Background:** Hepatic VOD causes morbidity and mortality in pediatric HPCT recipients with an incidence of 11-20%. Day+100 mortality rate is >80% with severe VOD. Optimal VOD prophylaxis is controversial. The combined effect of heparin and ursodiol in preventing VOD in pediatric patients remains unknown.

**Objectives:** The primary objective is to determine the incidence of VOD with heparin versus heparin and ursodiol prophylaxis in pediatric patients who underwent 1st HPCT.

**Design/Method:** A retrospective chart review of 529 patients was performed. VOD was defined by Baltimore criteria. VOD prophylaxis consisted of: heparin at 4 units/kg/hour beginning with conditioning through day+28 (group 1) or heparin through day+28 and ursodiol at 12 mg/kg/day through day+100 (group 2). VOD incidence was estimated with cumulative incidence (CI) curves considering death as competing event and Gray test was used for comparisons. Cox proportional hazards model was used to evaluate the effect of prognostic factors on probability of developing VOD.

**Results:** Patient characteristics are in Table1. CI of VOD at day+30 in group 1: 5.8% (95%CI:3.1-9.6%) and group 2: 2.5% (95%CI:1.1-4.6%) (p=0.0476). Regression analysis of patients who received alloHPCT (n=429) found age<1, use of busulfan and non-hematologic malignancies were significantly associated with VOD development. However, no influence of prophylaxis regimen on VOD incidence was found after adjusting for these risk factors (adjusted HR of VOD in group 2 vs 1:0.47, 95% CI:0.2-1.2%).

**Conclusion:** Low dose heparin and ursodiol prophylaxis appears to be an effective strategy in VOD prevention in pediatric patients. Future randomized prospective trials are needed.

Table 1: Patient characteristics by prophylaxis type.

Characteristics	Group 1: heparin n=207 N (%)	Group 2: heparin + ursodiol n=322 N (%)	P-value
<b>Age group (years)</b>			0.6272
0 - < 1	13 (6.3)	17 (5.3)	
≥ 1	195 (93.7)	305 (94.7)	
<b>Gender</b>			0.2139
Female	89 (43.0)	121 (37.6)	
Male	118 (57.0)	201 (62.4)	
<b>Diagnosis*</b>			0.0045
Hematologic malignancies	142 (68.6)	185 (57.5)	
Non-hematologic malignancies	34 (16.4)	50 (15.5)	
Non-malignant diseases	31 (15.0)	87 (27.0)	
<b>Year of transplant</b>			<0.001
1996 - 2001	170 (82.1)	1 (0.3)	
2002 - 2007	36 (17.4)	160 (49.7)	
2008 - 2013	1 (0.5)	161 (50.0)	
<b>Type of Transplant</b>			0.3473
Autologous	35 (16.9)	65 (20.2)	
Allogeneic	172 (83.1)	257 (79.8)	
<b>Type of Graft</b>			<0.0001
Bone Marrow	166 (80.2)	178 (55.3)	
PBSC	37 (17.9)	87 (27.0)	
Umbilical cord blood	4 (1.9)	56 (17.4)	
Bone Marrow + PBSC	0 (0.0)	1 (0.3)	
<b>CMV status (Donor/Recipient)</b>			0.0004
Negative/Negative	120 (61.0)	152 (48.7)	
Negative/Positive	20 (10.1)	59 (18.9)	
Positive/Negative	31 (15.7)	31 (9.9)	
Positive/Positive	26 (13.2)	70 (22.4)	
Missing	10	10	
<b>Conditioning Regimen</b>			<.0001
Cy, Bu or Bu/Cy (no TBI)	19 (9.7)	138 (43.1)	
TBI +/- Cy +/- other (no Bu)	156 (79.6)	135 (42.2)	
Other (no Bu, Cy or TBI)	21 (10.7)	47 (14.7)	
Missing	11	2	
<b>VOD</b>			0.0485
No	193 (93.2)	312 (96.9)	
Yes	14 (6.8)	10 (3.1)	

\*Hematologic malignancies: ALL, AML, CML, MDS, Lymphoma; Non-hematologic malignancies: solid tumors including brain tumors; Non-malignant diseases: SAA, Immunodeficiency, LCH, HLH; Cy - cyclophosphamide; Bu - Busulfan; TBI - total body irradiation;

# **ADOLESCENT AGE CONFERS HIGH RISK OF GRADE III AND IV TOXICITIES IN THE 30 DAYS POST-ALLOGENEIC STEM CELL TRANSPLANT: A RETROSPECTIVE COHORT STUDY OF PEDIATRIC PATIENTS UNDERGOING ALLOGENEIC STEM CELL TRANSPLANTATION FOLLOWING BUSULFAN-BASED CONDITIONING FOR MALIGNANT AND NON-MALIGNANT DISEASES**

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**Background:** In adults, high incidence of grade III-IV toxicities in the 30 days post allogeneic hematopoietic cell transplantation (alloHCT) is associated with higher transplant-related mortality (TRM) at 1 year. There remains a paucity of data on the incidence of grade III-IV toxicities in children and adolescents undergoing alloHCT.

**Design/Method:** Retrospective cohort study of 166 patients (0.1-22y) undergoing alloHCT between January 2000 and December 2013 for malignant and non-malignant diseases. Patients were conditioned on 1 of 3 Busulfan (Bu)-based conditioning regimens: reduced intensity (RIC): [Bu (6.4-8mg/kg) + Fludarabine (Flu) (150mg/m<sup>2</sup>)], reduced toxicity (RTC): [Bu (12.8-16mg/kg) + Flu (180mg/m<sup>2</sup>)] or myeloablative (MAC): [Bu (12.8-16mg/kg) + cyclophosphamide (120-200mg/kg) or melphalan (135mg/m<sup>2</sup>)]. Toxicities were scored using the CTCAE grading system.

**Results:** Median age at alloHCT was 8.5y (0.1-22y), malignant n=102, non-malignant n=64. Median number of grade III-IV toxicities in all groups was 3 (0-17). On univariate analysis, age  $\geq 12$  (p=0.002) was the single risk factor associated with increased incidence of grade III-IV toxicities in the 30 days post-transplant. Incidence of toxicities did not significantly differ between malignant and non-malignant groups, RIC v. RTC v. MAC regimens, by donor type, HLA match, primary disease or co-morbidity index. 1yr TRM in patients with below median (3) number of toxicities was 15.6% (p=0.007). A total of 59 pediatric patients received MAC regimens, n = 37. Of this cohort, 43% of patients <12y and 72.7% of patients  $\geq 12$  had above median number of grade III-IV toxicities (p=0.034). 1 year TRM was 10.8% for <12y and 22.7% for  $\geq 12$ y (p=0.272). RIC and RTC regimens were not associated with more than median toxicities in patients  $\geq 12$  yrs.

**Conclusion:** Despite advances in alloHCT, toxicity and organ impairment remain a significant cause of morbidity and mortality during the first year after transplant. Preliminary results suggest that higher incidence of grade III-IV toxicities in the 30 days post-alloHCT correlates with higher risk of TRM at 1yr. Age  $\geq 12$ y is significantly correlated with higher incidence of grade III-IV toxicities in the 30d post-transplant. Prospective studies to validate our finding along with approaches to decrease serious toxicities in adolescents are warranted.

PBMTC Poster 5023

## **THE EFFECT OF HEMODIALYSIS ON PLASMA DEFIBROTIDE PHARMACOKINETICS IN END-STAGE RENAL DISEASE PATIENTS: A PHASE 1, OPEN-LABEL STUDY**

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**Background:** Hepatic veno-occlusive disease (VOD/sinusoidal obstruction syndrome) is a potentially fatal complication of hematopoietic stem cell transplantation (HSCT). Severe VOD, clinically characterized by multi-organ failure (eg, pulmonary/renal), has been associated with >80% mortality. Defibrotide is approved in the European Union for the treatment of severe hepatic VOD in HSCT patients.

**Objectives:** To assess effects of hemodialysis on defibrotide pharmacokinetics (PK) in dialysis-dependent, end-stage renal disease.

**Design/Method:** In this open-label study, dialysis-dependent adults aged 18-80 years, with estimated glomerular filtration rate  $<15 \text{ mL/min/1.73 m}^2$ , received 2 doses of defibrotide 6.25 mg/kg via 2h IV infusions ( $\pm 10$  minutes): 1 dose on a nondialysis day (Day 1) and 1 on a dialysis day (Day 4). Four-hour dialysis began 1h into the 2h defibrotide infusion. Key PK parameters included area under the plasma concentration-time curve (AUC) from start of infusion (time 0) to time of last quantifiable plasma concentration following dosing ( $\text{AUC}_{0-t}$ ) and extrapolated to infinity ( $\text{AUC}_{0-\infty}$ ); maximum plasma concentration ( $C_{\max}$ ); time of  $C_{\max}$  ( $t_{\max}$ ); apparent terminal-phase half-life ( $t_{1/2}$ ); and systemic plasma clearance (CL). Point estimates and 90% confidence intervals (CIs) for log-scale differences were exponentiated for estimated ratios of geometric least-square (LS) means.

**Results:** The study included 6 patients. Key PK results on Day 1/Day 4 were  $\text{AUC}_{0-t}$ , ( $\mu\text{g}\cdot\text{h/mL}$ ) mean (coefficient of variation [CV] %): 102 (40.0)/111 (39.9);  $\text{AUC}_{0-\infty}$ , ( $\mu\text{g}\cdot\text{h/mL}$ ) mean (CV %): 103 (40.3)/114 (40.6);  $C_{\max}$  ( $\mu\text{g/mL}$ ), mean (CV %): 45.1 (35.1)/50.1 (38.1);  $t_{1/2}$  (h), mean (CV %): 0.712 (21.9)/0.967 (17.6); CL (L/h), mean (CV %): 5.87 (24.9)/5.38 (26.1);  $V_{ss}$  (L), mean (CV %): 6.34 (33.2)/6.90 (28.4);  $t_{\max}$  (h), median (min, max): 1.90 (1.50, 1.95)/1.78 (1.75, 1.95). The percent ratio of LS means (90% CI) following defibrotide dosing on Day 1/Day 4 were  $C_{\max}$ : 109.71 (97.23, 123.78);  $\text{AUC}_{0-t}$ : 108.39 (97.85, 120.07); and  $\text{AUC}_{0-\infty}$ : 109.98 (99.39, 121.70). One patient reported a possibly treatment-related adverse event (vomiting, mild severity).

**Conclusion:** The percent ratio of Day 4 (dialysis) to Day 1 (nondialysis) LS means and 90% CIs for AUC and  $C_{\max}$  were within the 80%–125% range. From these data, hemodialysis did not significantly affect defibrotide exposure/clearance in dialysis-dependent patients. The safety profile was consistent with previous studies.

PBMTC Poster 5024

### **TACROLIMUS AND Q8H MYCOPHENOLATE MOFETIL (MMF) PROPHYLAXIS IN CHILDREN AND YOUNG ADULT (CAYA) RECIPIENTS OF ALLOGENEIC STEM CELL TRANSPLANTATION (ALLOSCT) RESULTS IN A VERY LOW INCIDENCE OF GRADE II-IV ACUTE GVHD (AGVHD)**

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**Background:** Our previous pharmacokinetic study of MMF 900 mg/m<sup>2</sup> Q6H + tacrolimus for aGVHD prophylaxis in pediatric AlloSCT recipients suggested that 900 mg/m<sup>2</sup> Q8H was a more optimal MMF dosing regimen (Bhatia/Cairo et al. BBMT2010)

**Objectives:** To determine efficacy of q8h MMF plus tacrolimus for GVHD prophylaxis in CAYA AlloSCT recipients.

**Design/Method:** GVHD prophylaxis consisted of tacrolimus 0.03-0.04 mg/kg/day IVCI on Day -1 or 1st day of conditioning (target range 10-20 ng/mL) and MMF 900 mg/m<sup>2</sup> (max 1.5 g/dose) or 15 mg/kg (age > 18 y or weight > 70 kg; max 1.5 g/dose) IV/PO q8h starting on Day +1. MMF was discontinued on Day +30 or Day +60 in absence of aGVHD. In patients with non-malignant disorders, GVHD prophylaxis with both agents continued until Day +180. Mycophenolic acid (MPA) trough concentrations were obtained if MMF toxicity was suspected. aGVHD, chronic GVHD (cGVHD) and overall survival (OS) were determined by Kaplan-Meier method.

**Results:** Between 5/2011 – 9/2014, 41 patients (median age 12.5 years [range 0.1-23.5]; sex 28M:13F) received myeloablative (n=22) and non-myeloablative (n=19) conditioning for malignant (n=30) and non-malignant disorders (n=11). Donor sources were: 6/6 MSD (n=13), 4/6-6/6 UCB (n=16), and 9/10-10/10 MUD (n=12). Median time to myeloid and platelet engraftment was 16 and 34 days, respectively. Probability of Grade II-IV and grade III-IV aGVHD was 24.4% (CI95: 7.2-46.9) and 5.3% (CI95: 0-47.9), respectively. Probability of extensive and limited cGVHD was 20.7% (CI95: 3.6-37.9). In univariate analysis, myeloablative conditioning had 3.5 x greater risk of aGVHD compared to non-myeloablative (p=0.027; CI 0.83-14). There was no significant difference in aGVHD risk between donor sources, ATG/alemtuzumab use, CMV status, HLA match, or diagnosis. Probability of 1 year overall survival was 78.5% (CI95: 61.4-88.7). Several patients required MMF dose reductions in the late post-transplant period (beyond Day +30) for elevated MPA trough concentrations.

**Conclusion:** Tacrolimus plus q8h MMF prophylaxis resulted in a very low incidence of aGVHD in this group of CAYA AlloSCT recipients. The risk of aGVHD was higher in recipients of myeloablative conditioning. Further studies are necessary to determine optimal dosing and pharmacokinetics of MMF in the late post-transplant period.

PBMTC Poster 5025

## **HIGH DOSE CHEMO WITH STEM CELL RESCUE - A REVIEW OF ITS ROLE IN HIGH RISK SARCOMA**

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**Background:** Children with metastatic/relapsed Rhabdomyosarcoma (RMS) and Ewing sarcoma (ES) have a poor prognosis despite using high dose chemotherapy with stem cell rescue (SCT) in some cases. Recent results from the Euro EWING 99 trial for metastatic tumors reported EFS and OS of 27 ± 3% and 34% ± 4% respectively. CIBMTR data for RMS showed a PFS of 29% and an OS of 32% with a transplant related mortality of 5%. We reviewed our experience of SCT following completion of conventional chemotherapy in high-risk patients including disseminated and relapse sarcomas (ES and RMS).

**Objectives:** To evaluate clinical characteristics and outcome of pediatric patients with high-risk sarcoma undergoing autologous SCT.

**Design/Method:** Retrospective, chart review. Fourteen patients with high-risk sarcoma treated at Alberta Children's Hospital in Calgary Canada from 2007 to 2014 were analysed. Patients completed primary treatment including chemotherapy, radiation and surgery as per the institutional standards prior to consolidation SCT therapy.

**Results:** Four patients with ES were 14-17 years of age while 10 patient with RMS ranged in age from 2-14 years. 5 Patients underwent SCT for relapsed tumor. 10 patients had metastatic disease at diagnosis. 12 patients were in complete remission at the time of SCT. The conditioning regimens included Melphalan/Thiotepa for 8 patients, Busulphan/melphalan for 5 patients and Melphalan/Etoposide for 1 patient. The 5yr Event free survival was 50% (SE  $\pm$  25%)for ES and 50% (SE  $\pm$  15.8%) for RMS. The OS was 60%(SE  $\pm$  25%) for RMS and 50% (SE  $\pm$  15.6%)for ES. 1 patient with progressive RMS with isolated lung relapse after transplant remains alive. There was no statistical difference in survival based on size of tumor, metastasis, pre-transplant relapse or pre-transplant remission status. Treatment related mortality was not observed, however grade 3 hematologic toxicity, infections and mucositis were seen regularly. Grade 3 renal toxicity, veno-occlusive disease and hemorrhagic cystitis were seen in one patient each.

**Conclusion:** Myeloablative SCT therapy after completion of recommended protocol therapy may be beneficial in selected patients with high risk ES and RMS. The exact group of patients that benefit needs to be defined.

PBMTC Poster 5026

## **DEVELOPMENT OF POLYPS AS A LATE EFFECT AFTER HEMATOPOIETIC CELL TRANSPLANTATION (HCT)**

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**Background:** Advancements in the field of HCT have increased numbers of long-term survivors of childhood cancer. These survivors, however, are at risk for countless complications associated with their treatments, including secondary cancers. To date, screening for GI polyps has not been considered a recommended surveillance guideline for pediatric late-effects survivorship programs(1).

**Objectives:** We aimed to identify possible unifying characteristics of 5 children who developed GI polyps after HCT at Children's Hospital of Wisconsin. This information could help in recognizing potential risk factors for GI polyp formation and enhancing current screening procedures in long-term survivors of HCT.

**Design/Method:** Retrospective chart review.

**Results:** Between 2000-2013, five children with ALL (n=4) or AML (n=1) underwent HCT in CR1 (n=1) or CR2 (n=4) at a median age of 9 yrs (range, 16 mo – 16 yo). All patients underwent myeloablative conditioning regimens with TBI (range, 12-14 Gy). No patient had any preceding history of autoimmune disorders. One patient had family history of polyps; however, SMAD4 and BMPR1A mutations for juvenile polyposis were negative. Two patients presented with early infectious diarrhea (adenovirus at 1 month, rotavirus at 6 months). None developed H. pylori infections. Four patients developed acute gut GVHD (stage I, n=3; stage 3, n=1). One patient developed chronic gut GVHD. All 5 patients underwent endoscopy for evaluation of GI complaints, specifically bloody stool (n=3), chronic acid reflux (n=1), poor weight gain (n=1), and abdominal pain (n=2) at a median of 4.5 years (range, 9 mo – 5.75 yrs) after HCT. A median of 2 polyps (range, 1-11) per patient for a total of 26 polyps were discovered in the colon (n=20), stomach (n=5), and gastroesophageal junction (n=1). Two of the five patients had several polyps present with pre-malignant pathology (1/7 and 2/11 polyps, respectively). With a median follow-up of 13 yrs (range, 15 mo – 15 yrs), all patients are alive and well.

**Conclusion:** Although GI side effects are common after transplant, early screening for polyp formation is not part of recommended pediatric survivorship guidelines. Endoscopy should be considered for survivors with recurrent GI complaints. Multi-institutional studies can be used to confirm these observations.

1. Pulsipher BBMT 2012.

PBMTC Poster 5027

## **IS THERE A ROLE? OUTCOMES IN RELAPSED/REFRACTORY WILMS' TUMOR AFTER HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS HEMATOPOIETIC STEM CELL RESCUE**

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**Background:** Currently Wilms' tumors (WT) have cure rates approaching 90%, however prognosis in relapsed/refractory patients remains poor. Treatment for this group comprises a combination of surgery, radiation, standard chemotherapy or autologous hematopoietic stem cell rescue following high dose chemotherapy (HD-ASCR).

**Objectives:** To determine outcomes of HD-ASCR in patients with relapsed/refractory WT.

**Design/Method:** A retrospective chart review of all 24 patients with relapsed/refractory WT who were treated at the Ann and Robert H. Lurie Children's Hospital of Chicago with one or two cycles of HD-ASCR from 1992-2011. Initial cytoreduction consisted of 1) Cyclophosphamide, Carboplatin, Etoposide  $\pm$  Melphalan (n=14), 2) Cyclophosphamide, Thiotepa  $\pm$  Carboplatin (n=8), 3) Carboplatin, Thiotepa and Etoposide (n=1) or 4) Cyclophosphamide and Melphalan (n=1). All patients undergoing tandem transplants received 1) Melphalan and Cyclophosphamide (n=9), 2), Melphalan, Cyclophosphamide, Carboplatin and Etoposide (n=1) or 3) Melphalan alone (n=1) as cytoreduction.

**Results:** Median age at diagnosis was 5y and median time to HD-ASCR from diagnosis was 1.8y. Twenty patients relapsed, with median time to relapse 1.1y, and 4 were refractory. Initial histology was favorable in 20. Ten patients received one planned HD-ASCR. Eleven of 14 patients underwent planned tandem transplants; 3 received only one due to disease progression. At a median follow-up of 11.8y, 13 patients are alive, 7 of whom received tandem HD-ASCR. Transplant-related mortality (TRM) was low, with one death attributed to acute renal failure prior to engraftment. Thirteen patients relapsed after HD-ASCR, 10 of whom died of disease progression at a median of 0.8y and 3 are currently in remission. The 4-year event free survival (EFS) and overall survival (OS) are 40% and 59%, respectively. Age (p=0.2), gender (p=0.08), initial stage (p=0.07), histology (p=0.3), time to relapse from diagnosis (p=0.8) or disease status at ASCR (p=0.39) did not impact OS. Long-term morbidity in survivors included short stature (3), diastolic dysfunction (1), restrictive lung disease (1), gonadal toxicity (3), hypothyroidism (3) and atypical meningioma (1).

**Conclusion:** HD-ASCR is comparable to conventional chemotherapy with low TRM and acceptable morbidities in relapsed/refractory WT; however a larger multicenter cohort analysis is needed to determine its role.

PBMTC Poster 5028

## CT SCAN FREQUENTLY MISSES THE DIAGNOSIS OF POSTERIOR REVERSIBLE ENCEPHALOPATHY SYNDROME (PRES) AFTER STEM CELL TRANSPLANT

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**Background:** Posterior reversible encephalopathy syndrome (PRES) is a clinical syndrome characterized by vision changes, altered mental status, and seizures that are typically caused by acute rise in blood pressure and resolve over time. PRES has been reported after stem cell transplant (SCT) in association with hypertension from calcineurin inhibitors and steroids. The radiologic evaluation of PRES after SCT has not been well described.

**Design/Method:** A retrospective review of all cases of PRES in SCT recipients from January of 2004 to December of 2013 was performed. PRES was diagnosed if the patient developed encephalopathy, headache, seizures or visual disturbances with a CT and/or MRI showing imaging findings compatible with PRES.

**Results:** Twenty-two of 838 (2.6%) transplanted patients were diagnosed with PRES. PRES was diagnosed at a median of 49 days (IQR 31-88) after SCT. Nineteen patients (86%) presented with seizures, the other 3 (14%) had altered mental status. All patients underwent a brain CT and/or MRI, 21 of 22 patients (95%) received a CT scan when they became symptomatic, which was diagnostic of PRES in 8 of the 21 studies (38%). Eighteen patients (82%) received MRI, 17 of the 18 (96%) were consistent with PRES. The one MRI not consistent with PRES was done 20 days after initial diagnosis, subsequent to resolution of the abnormal CT findings. Notably in 13 patients initial CT scans did not demonstrate findings of PRES, which were subsequently found on MRI. The median time elapsed between CT and an MRI examination was 20 hours (range: 3.6 hours to 9 days).

**Conclusion:** CT scan serves as a good diagnostic test in emergency situations to rule out CNS bleed or infection in SCT patients with acute mental status changes or seizures, but it is not an adequate radiologic study for diagnosing of PRES after SCT. Patients with clinical symptoms suggestive of PRES, but negative CT, should undergo MRI of the brain after the acute event is controlled to assist in diagnosis. MRI results diagnostic of PRES would prompt physicians to provide good hypertension control and aid decision-making regarding invasive diagnostic procedures or intensification of empiric antimicrobial therapy.

PBMTC Poster 5029

## ELIMINATION OF THE POST-CRYOPRESERVATION WASH-STEP FOR UMBILICAL CORD UNIT TRANSPLANTATION

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**Background:** Umbilical cord blood (UCB) units are increasingly being used for allogeneic transplantation. Traditionally, UCB are thawed and washed in order to stabilize cells and to reduce potential toxicity secondary to DMSO; however, this process leads to significant cell loss.

We previously showed that the infused cell dose corrected for post-thaw viability is a strong predictor of neutrophil engraftment, but also that one third of cord blood cells are lost in the washing process (Hege et al., ASPHO/PBMTC 2013).

**Objectives:** To determine if preparation of UCB units by thawing alone or thawing followed by albumin-dextran dilution with elimination of centrifugation results in reduced cell loss while maintaining viability.

**Design/Method:** Seven cryopreserved UCB units were obtained for study, and two have been analyzed to date. Units were thawed and split into three fractions: (1) unmanipulated (thaw only), (2) 1:1 dilution with albumin-dextran (dilution only), (3) 1:1 dilution with albumin-dextran followed by centrifugation at 400xg (thaw-wash). TNC, CD34, and cell viability (trypan blue) were assessed. CFU cultures were established.

**Results:** Post-thaw cell recovery was similar between the thaw only, dilution only, and thaw-wash subsets, with an average TNC recovery of 98.5%, 101.5%, and 86.5%, and average CD34 recovery of 75.5%, 61%, and 67%, respectively. Cell viability showed a slight improvement within the thaw only and dilution only subsets over the thaw-wash with an average trypan blue viability of 98.5%, 92%, and 80% respectively. TNC corrected for viability demonstrates a similar trend of 90.5% for unmanipulated cells, 93.5% for dilution only, and 69.5% for thaw-wash. When viability was reassessed at 3 hours (estimated time from thaw to infusion), dilution-only units had greater viability: 56% for thaw only, 75% for dilution only, and 63.5% for thaw-wash fractions. CFU were plated, and results are pending at this time.

**Conclusion:** These preliminary data suggests that UCB thaw with albumin-dextran dilution without centrifugation results in improved cell recovery and viability with decreased manipulation. Thawed-only cells have lower viability at 3 hours, probably due to DMSO toxicity. These results will be confirmed with the remaining five cord blood units.

PBMTC Poster 5030

## **FAVORABLE OUTCOME OF VENO-OCCLUSIVE DISEASE WITH PREEMPTIVE AND AGGRESSIVE SUPPORTIVE CARE APPROACH FOLLOWING MYELOABLATIVE ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDREN**

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**Background:** Veno-occlusive disease (VOD) is a significant complication of hematopoietic stem cell transplantation (HSCT), particularly with myeloablative conditioning reaching a mortality rate of 84% in severe forms.

**Objectives:** To review the clinical course and the role of preemptive and aggressive supportive care in the outcome of VOD in myeloablative HSCT recipients.

**Design/Method:** Retrospective chart review of myeloablative allogeneic HSCT recipients from 2007 to 2014.

**Results:** Forty-nine transplants (ages 0.15 –22.9 years) with either intravenous busulfan (n=35; 71%) or total body irradiation (TBI)-based (n=14; 29%) myeloablative conditioning were

included. Busulfan doses were determined by pharmacokinetics targeting a median steady state concentration of 655ng/mL (603–800). The TBI range was 1200-1320cGy. Thirty-two cases (65%) received HSCT for malignant disorders. There were 16 cases (33%) of VOD; 12 (75%) following busulfan and 4 (25%) TBI-based conditioning. Five cases (31%) fulfilled the criteria for severe, 9 (56%) moderate, 2 (13%) mild VOD. Median age of the cases was 1.9 years (0.15–15.9) with and 9.3 years (0.5–22.9) without VOD. The median post-HSCT day for suspicion of developing VOD was 6 (1–13) due to weight gain (median 5.4%), hepatomegaly, and/or mild increase in serum bilirubin (median 0.9mg/dL) without fulfilling the established Seattle criteria. Aggressive fluid restriction, diuresis, and fresh frozen plasma (FFP) infusions were started with frequent assessments. Despite these measures, the patients went on to develop VOD by +11 days (7–16) with a median weight gain of 11.5% (2.1–25.7), hepatomegaly in 94%, median bilirubin of 2.7mg/dL (0.9–11.4), ascites in 81%, prolonged prothrombin time in 100%, and 8 (50%) had to be transferred to the intensive care unit staying an average of 7 (5–12) days. Two (13%) patients required oxygen supplementation and 1 underwent mechanical ventilation. All patients were on ursodeoxycholic acid; an average of 3 (1–5) different diuretics and 10 units (1–33) of FFP were used. One patient received 4 doses of defibrotide. Day +100 survival was 100% with complete resolution of VOD.

**Conclusion:** Preemptive and aggressive supportive care could provide favorable outcome in post-HSCT VOD and might have ameliorated the severity in our cases.

PBMTC Poster 5031

## **THE PHARMACOKINETICS OF DEFIBROTIDE IN NONDIALYSIS PATIENTS WITH SEVERE/END-STAGE RENAL DISEASE PATIENTS COMPARED WITH HEALTHY MATCHING SUBJECTS: A PHASE 1, OPEN-LABEL STUDY**

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**Background:** Hepatic veno-occlusive disease (VOD; also sinusoidal obstruction syndrome) is a potentially fatal complication of *hematopoietic* stem cell transplantation (HSCT). Severe VOD, clinically characterized by multi-organ failure (eg, pulmonary, renal), has been associated with >80% mortality. In the European Union, defibrotide is *approved for treatment of severe hepatic VOD post HSCT*.

**Objectives:** To compare plasma pharmacokinetics (PK) of defibrotide in nondialysis patients with severe/end-stage renal disease (ESRD) and healthy matching subjects.

**Design/Method:** This phase 1, open-label study enrolled nondialysis patients with severe/ESRD (estimated glomerular filtration rate [eGFR] <30 mL/min/1.73 m<sup>2</sup>), aged 18-80 years (Cohort 1) and matched healthy subjects with normal eGFR (Cohort 2). Both cohorts received defibrotide 25 mg/kg as 4 divided doses (6.25 mg/kg/dose) by 2-hour IV infusion q6h for 24 hours. PK parameters included area under the plasma concentration-time curve (AUC) from infusion start (time 0) to time of last quantifiable plasma concentration following dosing (AUC<sub>0-t</sub>), 6-hour length of the dosing interval (AUC<sub>τ</sub>), and extrapolated to infinity (AUC<sub>0-∞</sub>), and maximum

plasma concentration ( $C_{\max}$ ). Point estimates and 90% confidence intervals (CIs) were exponentiated to obtain estimates for ratios of geometric least-squares (LS) means.

**Results:** Each cohort included 6 patients. Key PK results after dose 1, Cohorts 1 and 2, respectively (% ratio of LS means [90% CI]) were  $C_{\max}$ : 53.6 and 39.6  $\mu\text{g/mL}$  (135.37 [105.06, 174.42]);  $\text{AUC}_{0-t}$ : 113.4 and 74.5  $\mu\text{g}\cdot\text{h/mL}$  (152.18 [117.60, 196.94]);  $\text{AUC}_{0-\infty}$ : 114.6 and 74.9  $\mu\text{g}\cdot\text{h/mL}$  (153.01 [117.70, 198.91]). The corresponding values after dose 4 were  $C_{\max}$ : 52.6 and 38.0  $\mu\text{g/mL}$  (138.34 [106.05, 180.46]);  $\text{AUC}_{0-t}$ : 108.9 and 68.3  $\mu\text{g}\cdot\text{h/mL}$  (159.55 [118.15, 215.47]);  $\text{AUC}_{\tau}$ : 109.0 and 68.4  $\mu\text{g}\cdot\text{h/mL}$  (159.36 [118.11, 215.05]). Half-life in Cohorts 1 and 2 were 0.725h and 0.562h after dose 1, and 0.498h and 0.217h after dose 4, respectively. No adverse events were reported.

**Conclusion:** Defibrotide exposure was higher in severe/ESRD nondialysis patients than in healthy matching controls after single and multiple doses. PK exposure parameters after multiple doses were within 5%-8% of exposure parameters after the first dose in both cohorts indicating no accumulation, consistent with defibrotide's short half-life versus dosing interval. Safety profile was consistent with previous studies.

PBMTC Poster 5032

## BIOPSY IDENTIFIES CD20+ B CELLS AS POTENTIAL MEDIATORS OF MINIMAL CHANGE NEPHROTIC SYNDROME CHRONIC GVHD

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**Background:** Acute kidney injury after hematopoietic stem cell transplantation (HSCT) is common, but renal chronic graft versus host disease (cGVHD) is not frequently reported. Nephrotic syndrome (NS) is a rare and uncommon manifestation of cGVHD, especially in pediatrics, but most often presents as membranous nephropathy.

**Objectives:** We report two cases of renal GVHD manifesting as minimal change disease NS.

**Design/Method:** Retrospective chart review of two patients diagnosed with NS after HSCT.

**Results:** Patient 1 is a 14 year old African American male who underwent 5/6 matched double cord blood transplant for h/o multiply relapsed acute lymphoblastic leukemia. Cyclosporine (CSA) was used for GVHD prophylaxis but discontinued at day +60 due to development of leukoencephalopathy. Within weeks of discontinuation of CSA, patient presented with clinical picture of NS. Renal biopsy was consistent with minimal change NS. Special immunostains were strongly positive for CD20. Patient 2 is a 5 year old Caucasian female with secondary AML after treatment for neuroblastoma who underwent 10/10 matched unrelated transplant with busulfan, cyclophosphamide and ATG as preparative regimen. CSA was used for GVHD prophylaxis and discontinued at day +180. Two weeks after discontinuing immunosuppression, patient developed clinical syndrome of NS. Renal biopsy showed minimal change NS. Immunostains were also positive for CD20 but less than patient 1. Both patients were initially managed with intermittent hemodialysis as steroid-sparing treatment with clinical improvement. Patient 1 was also treated with a single course of rituximab and prednisone at 2mg/kg/day with rapid taper. His symptoms



completely resolved, and he has been in remission from NS and leukemia for 4 years. Patient 2 was started on tacrolimus in addition to steroids and weaned off steroids over 6 months and has been in remission from NS and AML.

**Conclusion:** NS is a rare manifestation of cGVHD, and minimal change disease is less common than the more frequent membranous form. We present data to suggest that the pathophysiology includes B-cell dysregulation, further supported by our patient's response to B-cell depletion with rituximab. Biopsies of rare cGVHD manifestations may also guide treatment decisions.

PBMTC Poster 5033

## **EOSINOPHILIC MENINGOENCEPHALITIS AND PROTRACTED COMA AS A PRESENTATION OF SEVERE QUIESCENT CHRONIC GVHD IN A PATIENT AFTER HEMATOPOIETIC STEM CELL TRANSPLANT: CASE REPORT & REVIEW OF THE LITERATURE**

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**Background:** Meningoencephalopathy is a rare, poorly characterized complication of hematopoietic stem cell transplant (HSCT). In general less than 2% of all patients with meningoencephalopathy have CSF eosinophilia (>10% eosinophils). A case-series suggests an incidence of meningoencephalopathy of 6% after allogeneic HSCT, with causes including infection, organ failure, medications, and rarely tumor, with 19% remaining of unknown origin despite investigation.

**Objectives:** We present a case of a 6 year-old male with a history of M5 AML and HSCT course notable only for acute graft-versus-host-disease (GVHD) of liver and skin, which had resolved with minimal tacrolimus. He presented 224 days after 8/8 matched related HSCT with fever, vomiting and malaise, positive sick contacts with viral disease, and rapidly developed meningoencephalitis with generalized seizures.

**Design/Method:** Multiple CSF studies revealed pleocytosis with an eosinophilia (26%) and high protein level (297 mg/dL), with normal peripheral blood counts except eosinophilia (3,770 cells/mcL). He developed cerebral edema, requiring emergent intubation and EVD, while concurrently being treated with multiple anti-microbials for bacteria, tuberculosis, viruses, fungi and parasites, as well as steroids, with improvement in cerebral edema and EVD removal. He was subsequently extubated but remained neurologically devastated without withdrawal to pain.

**Results:** CSF studies, including flow cytometry, paraneoplastic panel, and mycobacteria, viral, bacterial, and fungal cultures and PCR tests were negative. Repeat MRIs showed worsening diffuse hyperintense signal on T2-weighted and T2 FLAIR in cerebral and cerebellar white matter. He developed diffuse diarrhea as well as elevated liver enzymes and an oral ulcer (HSV negative). Liver and GI biopsies showed inflammatory hepatitis and few apoptotic bodies, respectively, suggestive of chronic GVHD (cGVHD). After few days of steroid and tacrolimus therapy, liver enzymes and bilirubin improved and patient was extubated with improved pupil response, suggesting improved neurological status.

**Conclusion:** Using the 2014 Consensus criteria for cGVHD diagnosis and staging, he has severe quiescent, overlap cGVHD (scores of 3 for liver and CNS) that presented with eosinophilic meningoencephalitis. While eosinophilia is commonly associated with cGVHD, eosinophilic

meningitis in the absence of other cGVHD manifestations has not been reported and should now be considered a possible presentation of severe cGVHD

PBMTC Poster 5034

**HIGH DOSE CHEMOTHERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION (HDC-ASCT) AND PHARMACOKINETIC ANALYSIS OF ULTRAFILTERABLE PLATINUM (UF-PT) DURING SLED IN A PATIENT WITH RECURRENT WILMS TUMOR AND RENAL FAILURE**

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**Background:** Wilms Tumor (WT) is the most common pediatric renal tumor, with current survival rate of 90%. However, treatment of high risk recurrent WT remains a challenge and HDC-ASCT is a potentially life-saving option.

**Objectives:** Most patients with relapsed WT will develop chronic kidney disease, due to nephrectomy and prior therapies. HDC-ASCT in a child with chronic renal failure (CRF) presents a special challenge to tailor the dose and timing of HDC and dialysis to eradicate the tumor without excessive toxicity. ASCT in hemodialysis-dependent (HDD) children for recurrent WT is rare, and pharmacokinetic data in these patients is sparse. The optimal conditioning regimen is unknown. We are aware of only one report of HDC-ASCT in a HDD WT patient, in whom pharmacokinetic data for carboplatin (CPT) was evaluated\*. Recurrence of tumor was detected in that patient 194 days after ASCT.

**Design/Method:** We present a 9 year old HDD male with recurrent WT who underwent HDC-ASCT. He developed stage IV favorable histology WT at age 4, underwent nephrectomy, chemotherapy and radiation. WT recurred 4 years later in the operative bed, with multiple liver metastases. He received 4 courses of salvage chemotherapy, and developed CRF after the first course. His tumors responded and he was rendered near CR with surgery. HDC pre-ASCT included Etoposide 250 mg/m<sup>2</sup> daily and CPT 250 mg/m<sup>2</sup> daily on days -8 to -6, and cyclophosphamide 50 mg/kg daily on days -5 to -3. He received Sustained Low Efficiency Dialysis (SLED) during and 10 hours after each dose of CPT (SLED intensity varied), and conventional HD 6 hours after cyclophosphamide and again on day -1. UF-PT pharmacokinetics were measured.

**Results:** UF-PT levels were: pre-SLED (mg/L) 26.8-26.9, post-SLED 1.5-4.5; T<sub>1/2</sub>: 88-138 min; AUC: 2960-4910 mgXmin/L, similar to myeloablative target values for normal renal function. Toxicity included moderate mucositis and ileus, both resolved, without ototoxicity. Engraftment of neutrophils was day +18 and platelets was day +49. He continued in CR on HD, but otherwise was clinically well until he developed tumor recurrence at 357 days post ASCT.

**Conclusion:** HDC-ASCT is feasible and has potential benefit in WT patients on HD.

\*Dagher, JPHO 1998

This work was conducted at Kosair Children's Hospital, Louisville, Kentucky, USA.

## ENDOCRINOPATHIES AND BONE HEALTH IN FANCONI ANEMIA PATIENTS FOLLOWING HEMATOPOIETIC CELL TRANSPLANTATION

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**Background:** Endocrinopathies and low bone mineral density (BMD) have been described after hematopoietic cell transplantation (HCT), but data are limited in Fanconi Anemia (FA) patients.

**Objectives:** To study the prevalence and risk factors for endocrinopathies and low BMD in FA patients following HCT.

**Design/Methods:** This is a retrospective review of 44 FA patients who underwent alternative donor [HLA-mismatched related (n=4) or unrelated (n=40)] HCT for aplastic anemia at the University of Minnesota between 2006 and 2013 and survived >1 year. Patients received total body irradiation 300 cGy, Cyclophosphamide 40 mg/kg, Fludarabine 140 mg/m<sup>2</sup> and ATG, followed by T cell depleted marrow (n=30) or cord blood (n=14). Median age at transplant was 8.9 years (range 3.3-34.4). Median follow up was 3.1 years (range 1-8). We evaluated hypothyroidism, hypergonadotropic hypogonadism, short stature, vitamin D deficiency and BMD by dual energy x-ray absorptiometry. Risk factor assessment included age at transplant, gender, GVHD and complementation group.

**Results:** 91% of patients had at least one, and 57% had three or more endocrinopathies. Hypothyroidism was seen in 57%, hypergonadotropic hypogonadism in 20%, short stature in 50%, vitamin D deficiency in 71%, and low BMD in 24%. Short stature and low BMD were associated with a younger age at transplant. Gonadal failure was associated with female sex and GVHD. (See Table 1)

**Conclusion:** Endocrinopathies and low BMD are common in FA patients following HCT. We recommend assessment prior to transplant and close follow up after transplant to better understand the role of transplant in endocrinopathies.

Table 1

	Hypothyroidism Φ	Hypergonadotropic Hypogonadism ζ	Short Stature Υ	Vitamin D Deficiency φ	Low Bone Mineral Density Δ
n <sup>∅</sup>	44	30	44	42	41
Age at transplant (yrs)					
<10	17/29 (58.6)	1/15 (6.7)	18/29 (62.1)	21/28 (75.0)	7/28 (25.0)
10-16	5/8 (62.5)	3/8 (37.5)	4/8 (50.0)	6/8 (75.0)	3/7 (42.9)
>16	3/7 (42.9)	2/7 (28.6)	0/7 (0)	3/6 (50.0)	0/6 (0)
p-value	0.47	0.26	0.01	0.44	0.07
Gender					
Male	13/24 (54.2)	0/15 (0)	13/24 (54.2)	16/23 (69.6)	4/22 (18.2)
Female	12/20 (60.0)	6/15 (40.0)	9/20 (45.0)	14/19 (73.7)	6/19 (31.6)
p-value	>0.99	0.02	0.76	0.99	0.47
GVHD					
Yes	4/6 (66.6)	3/6 (50.0)	3/6 (50.0)	3/5 (60.0)	1/5 (20.0)
No	21/38 (55.3)	3/24 (12.0)	19/38 (50.0)	27/37 (73.0)	9/36 (25.0)

<sup>Ψ</sup> Treatment with thyroid hormone at the time of examination, free T4 of <0.7 ng/dL, or TSH of >4.0 mIU/L

<sup>ζ</sup>Females: FSH of >40 and >10 years of age, Males: LH >10, low testosterone, and >11 years

<sup>Y</sup> Height less than 2 standard deviations below the mean height for age

<sup>φ</sup> 25-hydroxyvitamin D level less than 30 µg/L

<sup>Δ</sup> Height adjusted Z score of less than -2, measured for total body and lumbar spine (L1-L4) or femoral neck if >20 years old

∂n= number of patients with available data.

PBMTC Poster 5036

## **A UNIQUE CASE OF CHRONIC GVHD WITH BRONCHIOLITIS OBLITERANS AND MEMBRANOUS BRONCHIAL WEBS AFTER BONE MARROW TRANSPLANT**

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**Background:** Bronchiolitis obliterans (BO) is a rare but serious complication associated with chronic graft versus host disease (cGVHD) after bone marrow transplant (BMT). Typically, BO is defined as narrowing of the small airways secondary to inflammation. The formation of membranous webs in larger airways in association with BO has been reported in patients after lung transplant but has not previously been described after BMT.

**Objectives:** To report a case of severe respiratory failure secondary to the formation of diffuse pulmonary membranous webs in a patient with cGVHD after a matched-related-donor transplant for sickle cell disease.

**Design/Method:** Case Report

**Results:** The patient is an 11 year old female who received a matched related donor transplant for sickle cell disease and recurrent acute chest syndrome. She developed cGVHD of the mouth, eyes, skin and lungs seven months post-transplant, coinciding with significant decline in her pulmonary function (FVC dropped from 80 to 57% and FEV1 91 to 56% of predicted). Chest CT showed consolidation and ground glass opacities concerning for infection versus pulmonary GVHD. Despite prolonged treatment with broad-spectrum antibiotics and immunosuppressive therapy, her pulmonary function continued to decline, and after 10 months she had an FVC 32% and FEV1 20% of predicted. During this time period, she had three bronchoscopies performed that were negative for all viral, fungal and bacterial studies except for normal respiratory flora and rhinovirus/enterovirus PCR. Seventeen months post-transplant, she developed respiratory failure requiring intubation. Bronchoscopy revealed multiple intraluminal membranes that occluded the airways completely, starting at the level of the sub-segmental bronchioles bilaterally. Pathology of the webs showed a fibrinous structure. Though treatment was attempted with manual disruption of the membranes, direct instillation of tPA and administration of nebulized tPA, the patient's condition worsened and ultimately led to progressive hypercapnia and death.

**Conclusion:** We present here a unique case of membranous obliterative bronchiolitis in the setting of cGVHD after BMT. There have been no previously reported cases of this phenomenon occurring in patients post BMT, though it has been seen after lung transplant. Understanding the connection between these conditions may help elucidate their etiology and potential treatment options.

## **DECLINE IN DONOR LYMPHOID CHIMERISM PRECEDING ACUTE MYELOID LEUKEMIA RELAPSE FOLLOWING ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION CONDITIONED WITH BUSULFAN AND FLUDARABINE**

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**Background:** Busulfan-Fludarabine (BuFlu) conditioning has been utilized with reduced toxicity in allogeneic hematopoietic stem cell transplantation (HSCT) for acute myeloid leukemia (AML). However, concerns of slower donor chimerism achievement and risk of graft failure with BuFlu regimen have been raised.

**Objectives:** To report on our observation of evidence for decreasing donor chimerism precedes relapse in acute myeloid leukemia following BuFlu-conditioned allogeneic HSCT.

**Design/Method:** Retrospective chart review of AML cases whom underwent myeloablative HSCT conditioned with BuFlu was conducted and bone marrow (BM) and/or peripheral blood (PB) donor chimerism by short tandem repeat (STR) analysis and minimal residual disease (MRD) status assessment by flow cytometry were investigated.

**Results:** Seven children were included in the analysis. Intravenous busulfan doses were determined by pharmacokinetics targeting a steady state concentration 500-800ng/mL and total Fludarabine dose was 200mg/m<sup>2</sup>. Tacrolimus and mycophenolate mofetil were used for graft-versus-host disease prophylaxis. Four were in second complete remission, one in the first and two had disease at HSCT. Donor sources were all matched; unrelated BM in 3, unrelated cord blood in 2, sibling BM in 1 and sibling PB in the last. One experienced primary graft failure and one very early relapse by day +13. Median engraftment was on day +14 in the others. Two became long-term survivors (28.5%). One patient had isolated extramedullary relapse on day +76. In the remaining two cases, one had decreased PB lymphoid donor chimerism (LDC) from 87% on day +32 with negative MRD to 42% on day +42 with demonstration of relapse on day +61 when LDC was down to 27%. The second patient had immunosuppression withdrawal soon after engraftment that was associated with increase in LDC and decline in MRD leading to overlap syndrome. Lymphoid donor chimerism was 50%, 84%, 95, and 100% and MRD 0.06%, 0.04%, 0.7%, and 0.02% on days + 14, +25, +32, +66, respectively. On day +286, LDC dropped to 93% when he experienced relapse.

**Conclusion:** Declining LDC indicating impending graft loss may precede AML relapse in HSCT recipients conditioned with BuFlu. Frequent STR and MRD analysis may help identify such cases and prompt therapeutic intervention.

## **NOVEL T CELL DEPLETION TECHNIQUES IN PAEDIATRIC HAPLOIDENTICAL**

## STEM CELL TRANSPLANTS: THE MONEY EQUATION

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**Background:** Novel ex-vivo T cell depletion techniques increase the cost of haploidentical stem cell transplantation significantly. However this is offset by the ready availability of a stem cell source and decreased expenditure towards stem cell procurement which costs approximately USD 44,000 for NMDP registry. Early engraftment reducing blood product requirement, decrease in the incidence of graft versus host disease (GVHD) and early NK cell reconstitution can be exploited by a reduction in the length of hospital stay and medication expenditure.

**Objectives:** To analyze different ex vivo T cell depletion techniques used in our hospital from cost perspective.

**Design/Method:** We describe haploidentical transplant in two children using different T cell depletion methods and the cost involved.

**Results:** A 16 year old male with refractory undifferentiated leukaemia achieved remission after FLAG (fludarabine, cytarabine, GCSF) chemotherapy. Haploidentical peripheral blood stem cell (PBSC) transplantation was performed using mother (KIR mismatched and KIR B haplotype). GCSF mobilized PBSCs were collected and processed over two days by negative selection for CD3. *In vivo* B cell depletion was done with Rituximab (375mg/m<sup>2</sup>) on D-1. The total cost of CD3 depletion was USD 15,500 equivalent compared to the quoted cost for TCRαβ and CD19 depletion (USD 35,000 equivalent). He had rapid early engraftment but with cytomegalovirus and EBV reactivation successfully treated with antivirals and Rituximab. He is currently 10 months post transplant remains well with full donor chimerism. Case 2: An infant with Hoyerall Hreidarsson syndrome was transplanted at 10 months of age for severe marrow failure. Father was the PBSC donor (KIR mismatched, KIR B haplotype). A single day's stem cell collection was processed for TCRαβ and CD19 depletion. The total procedure related cost was USD 17,500 equivalent. Rapid early engraftment without any significant post transplant complications was noted. He is now 15 weeks post transplant with no evidence of infections or GVHD.

**Conclusion:** Ex vivo T depleted Haploidentical stem cell transplantation can be cost effective as compared to unrelated donor or cord transplant when procurement cost, length of hospital stay, blood component therapy and avoidance of post transplant immunosuppressive medications are included. This potentially can make it a financially attractive package in the resource limited and emergency setting.

PBMTC Poster 5039

## THE SUCCESSFUL TREATMENT OF RECURRENT CNS DISEASE POST-HCT IN PATIENTS WITH HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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**Background:** Hemophagocytic lymphohistiocytosis (HLH) is a rare hyperinflammatory syndrome that affects multiple organs, including the central nervous system (CNS). CNS involvement occurs in 37-73% of patients and is associated with higher mortality rates than patients without CNS involvement. Current standard of care for CNS disease utilizes high dose dexamethasone therapy and intrathecal methotrexate and hydrocortisone. However, the morbidity and mortality rate for patients with CNS involvement remains high. There is limited literature on treatment and management strategies for CNS involvement post-HCT.

**Design/Method:** We conducted a single-center retrospective case series to describe the clinical course of five patients (age 2-17 mo) diagnosed with genetic HLH (PRF1 (n=2), Munc 13-4 (n=3)) who underwent HCT (UCB (n=4), MUD (n=1)) with myeloablative busulfan-based conditioning regimens (BuCYATG (n=4) BuFluATG (n=1)) and had CNS involvement post-HCT. Four patients had CNS involvement pre-HCT. GVHD prophylaxis consisted of CSA and MMF. All patients received a graft from an unrelated donor (UCBs: HLA match 5/6 (n=2), 6/6 (n=2); MUD: HLA match 10/10 (n=1)) with a median TNC dose of  $3.28 \times 10^7/\text{kg}$  (range  $1.46 \times 10^7/\text{kg}$ — $18.9 \times 10^7/\text{kg}$ ) for UCB transplants. All patients were serially monitored with monthly surveillance lumbar punctures (LPs) prior to developing neurologic symptoms and then treated with systemic dexamethasone for CNS disease.

**Results:** Systemic dexamethasone controlled CNS disease as a single agent in all five patients post-HCT. In three of the cases, CNS relapse was caught by routine LPs, but before the clinical appearance of symptoms. All patients are alive, a median of 29 months post-transplant (range 12-60 months). Four patients have mild neurological deficits, including mild speech delay (3) and one patient who exhibited brainstem herniation on day 0, due to CNS HLH, has made a substantial recovery of function with residual deficits of focal weakness on the right side. One patient has no deficits.

**Conclusion:** This case review supports vigilant screening of LPs post-transplant for occult CNS disease prior to the development of symptoms and the use of systemic dexamethasone for control of disease in this patient population. Future prospective clinical trials are needed to further evaluate this strategy.

PBMTC Poster 5040

## **BENEFIT OF RECOMBINANT ACTIVATED FACTOR VII IN POST-ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION COMPLICATIONS WITH INTRACTABLE BLEEDING**

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**Background:** Severe gastrointestinal (GI) bleeding in gut graft versus host disease (GvHD) and diffuse alveolar hemorrhage (DAH) are associated with significant mortality in allogeneic hematopoietic stem cell transplantation (HSCT) recipients. In addition to immune response

modifying therapies, supportive measures are used to control active bleeding with limited success.

**Objectives:** To report the experience of recurrent and/or prolonged recombinant activated Factor VII (rFVIIa) therapy in severe bleeding complications following allogeneic HSCT.

**Design/Method:** Retrospective review of medical records was conducted in three allogeneic HSCT recipients for whom rFVIIa was used in a recurrent and/or prolonged manner for intractable bleeding.

**Results:** All three patients received various immune response-modifying therapies throughout treatment. Adequate transfusion support with fresh frozen plasma, cryoprecipitate and platelets, as well as vitamin K and octreotide were provided in gut GvHD cases prior to initiation of rFVIIa treatment. Patient 1 developed DAH following matched sibling donor HSCT for leukocyte adhesion defect. He developed three episodes of DAH on days +14, +25, and +67. He received rFVIIa infusions for refractory bleeding in the second and third episodes totaling 10 doses with cessation of pulmonary bleeding in 3 days. He is currently 7.4 years from HSCT. Two children with gut GvHD were transplanted for refractory anaplastic large cell lymphoma (patient 2) and congenital amegakaryocytic thrombocytopenia (patient 3) using matched unrelated donor and mismatched umbilical cord blood cells, respectively. Both developed acute GvHD of the gut, skin, and liver and started significant GI bleeding by day +23. Due to life-threatening bleeding, they were given rFVIIa with improvement in bleeding during acute episodes; rFVIIa therapy was tapered or discontinued and resumed upon recurrence or worsening. A total of 265 doses of rFVIIa were used in patient 2 over 54 days. She died of recurrent disease. Patient 3 received 82 doses rFVIIa over 26 days. He is +5.2 years post HSCT. Use of rFVIIa was associated with decreased packed red blood cell transfusion requirements and intensive care unit days.

**Conclusion:** Use of rFVIIa can provide temporary benefit in severe bleeding episodes associated with DAH and gut GvHD, if other measures are not successful.

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## **SPONTANEOUS SUBARACHNOID HEMORRHAGE (SAH) DURING PERIPHERAL BLOOD PROGENITOR CELL (PBPC) INFUSION IN A PEDIATRIC PATIENT WITH ACUTE MYELOID LEUKEMIA (AML)**

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**Background:** The infusion of either bone marrow or PBPCs is a routine procedure performed at bedside. Bone marrow or PBPC products are generally used fresh and infused slowly through a central vein. Most common adverse effects include nausea, vomiting, allergic reaction, hypotension, hypertension, tremor, fever, chest pain, cardiac alterations, dyspnea, and abdominal cramps. To our knowledge, spontaneous SAH has not been reported as a complication of PBPC infusion.



**Objectives:** Describe spontaneous SAH as a potential complication of PBPC infusion.

**Design/Method:** Case Report

**Results:** 8 year old African American male with CNS negative high-risk AML (monosomy 7) in first complete remission (CR-1) underwent 6/8 matched cord blood transplant after conditioning regimen of busulphan, fludarabine, and anti-thymocyte globulin (ATG). Post-transplant course was complicated by CMV viremia, and engraftment failure. The patient underwent a second transplant fifty-five days from his initial transplant, conditioned with fludarabine, ATG, and 400cGy TBI. Premedicated with acetaminophen, diphenhydramine, and methylprednisolone as per the institutional standard protocol, he received 9/10 matched (antigen A mismatch) unrelated donor peripheral blood stem cells with a cell dose of  $5.16 \times 10^6$  CD34+ cells/kg in 110mL. The patient received 100mL over fifty minutes without any significant events. However, nearing completion of stem cell infusion, patient suffered a one minute self-limiting right-sided complex-partial seizure. Post-seizure CT of the brain revealed a SAH along the falx and medial aspect of the bilateral hemispheres. MRI/MRA revealed no underlying vascular malformations. PT/INR and PTT were normal. Pre-PBPC infusion predisposing factors included a 5mL/kg platelet transfusion 10 hours prior for platelet count of 10K/MM3, persistent systolic and diastolic blood pressures >99th percentile for 8 hours leading up to and during the infusion, pre-transplant conditioning regimen, and underlying diagnosis of AML. Following the event, blood pressures normalized and he remained seizure-free on anti-hypertensive and anti-epileptic medications. No further CNS bleeding was noted on serial CT imaging maintaining the platelet count >50K/MM3. Two month follow-up imaging showed complete resolution of SAH.

**Conclusion:** Spontaneous SAH is a potential consequence of PBPC infusion, particularly in the context of other predisposing factors. Awareness of such a life-threatening complication is paramount in its early recognition and prevention.