YOUNG INVESTIGATOR AWARD PRESENTATION

Pen inactivation promotes loss of MYC "oncogene addiction" in a conditional zebrafish model of T-ALL

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Background: Some but not all experimental MYC-induced tumors are dependent on ongoing MYC activity for maintenance of the tumor phenotype, a phenomenon known as "oncogene addiction". Despite recent advances, the genetic determinants of addiction to the MYC oncogene remain incompletely understood.

Objectives: The objective of this study was to test the hypothesis that the Pen-PI3K-Akt pathway is an important in vivo determinant of oncogene addiction to MYC in a conditional zebrafish model of T-ALL, a hypothesis that has not previously been tested in any experimental system.

Design/Method: Zebrafish harboring germline Pen loss-of-function mutations were mated into the double-transgenic rag2:Myc:ER:rag2:GFP zebrafish line, and T-ALL onset and regression were monitored via GFP fluorescence as detailed below. To determine whether Akt activation phenocopies Pen loss, we injected a rag2:myr-Akt2 transgene into zebrafish from the rag2:Myc:ER line at the 1-cell stage, and monitored T-ALL onset and regression as below.

Results: We have developed a conditional zebrafish model of MYC-induced T-ALL in which the majority of tumors are dependent on ongoing MYC:ER transgene activation by hydroxyurea (4HT) treatment. We mated zebrafish harboring loss-of-function Pen mutations into our rag2:Myc:ER:line, offspring were raised in 4HT, and T-ALL onset was monitored via fluorescence microscopy. At the time of T-ALL onset, fish were removed from 4HT, and tumor regression was monitored weekly. T-ALL regression occurred in 69% of 39 pen wild-type zebrafish, while regression occurred in only 27% of Pen zebrafish harboring loss of at least one pen allele (p = 0.005). The effect of pen inactivation on T-ALL regression was phenocopied by the expression of a constitutively active murine Akt2 transgene in the MYC:ER line, in which tumor regression occurred in 14% of 21 MYC:ER zebrafish injected with a constitutively active rag2:myr-Akt2 transgene, versus 75% of 12 rag2:GFP-injected controls (P = 0.0009).

Conclusion: Pen inactivation promotes loss of "oncogene addiction" to MYC in our zebrafish model of T-ALL, an effect that is phenocopied by expression of a constitutively active Akt transgene. Our findings suggest that constitutive signaling through the PI3K-Akt pathway overrides the requirement for ongoing MYC transgene activation in zebrafish T-ALL lymphoblasts.

CELL-SPECIFIC GENE EXPRESSION IN LCH: BACK TO HISTIOCYTOSIS X?

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Background: Langerhans Cell Histiocytosis (LCH) is a rare disease characterized by heterogeneous lesions containing CD207+ Langerhans cells and lymphocytes that can arise in almost any tissue and may cause significant morbidity and mortality. After decades of research, the pathogenesis of LCH remains speculative.

Objectives: This cell-specific gene expression study was performed to test the prevailing model of LCH pathogenesis that lesions arise due to malignant transformation of epidermal Langerhans cells.

Design/Method: Data was collected from 15 LCH biopsy samples, 12 control skin samples and 7 LCH peripheral blood samples, and cells were isolated using flow cytometry (purity > 95%). LCH CD207+ cells were isolated from LCH lesions, control Langerhans cells (LC) were isolated from normal skin, quality of RNA was verified, RNA was amplified, and gene expression was compared using Affymetrix U133A Plus 2.0 chips, then analyzed with the Significance Analysis of Microarrays (SAM) method. Verification was done using qPCR. According to our data, we have identified 2113 differentially-expressed genes (FDR < 0.01), 1297 were up-regulated and 816 were down-regulated.

Results: Some but not all genes identified in this study were also identified in other LCH studies. Up-regulated genes included ITGA4 (CD49d), ANPEP (CD13) and CD300LF. Compared to the peripheral control LCs in our study, however, several novel genes whose products activate and recruit T cells to sites of inflammation, including SPP1 (osteopontin), were highly over-expressed in LCH CD207+ cells. Furthermore, several genes associated with immature myeloid dendritic cells were over-expressed in LCH CD207+ cells, including ITGAX (CD11c), ITGAM (CD11b), ICAM1 (CD54), CD33, CD1d, ITG44 (CD49d), ANPEP (CD13) and CD300LF. Compared to the peripheral CD3+ cells from LCH patients, the LCH lesion CD3+ cells yielded only 162 differently-regulated genes (FDR < 0.01), and the expression profile of the LCH lesion CD3+ cells was consistent with an activated regulatory T cell phenotype with increased expression of FOXP3, CTLA4 as well as SPP1.

Conclusion: Results from this study support a model of LCH pathogenesis in which lesions do not arise from epidermal Langerhans cells, but from accumulation of bone marrow-derived immature myeloid dendritic cells that recruit activated lymphocytes.

2010 ASPHO ABSTRACTS

PLENARY PLATFORM SESSION 300A

Hydroxyurea treatment of infants with sickle cell anemia: Results of the Baby Hug Study

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Background: Although hydroxyurea (HU) decreases vaso-occlusive events (VOE) in adults and children with sickle cell anemia (SCA), it has not been demonstrated to prevent organ damage or reduce acute complications in infants.

Objectives: Here we report the results of the Phase III double-blind placebo-controlled trial designed to determine if HU treatment is safe, protects spleen and kidney function, and improves clinical and laboratory findings.

Design/Method: Subjects with HbSS or HbSβthalassemia age 9-17 months at entry received liquid HU, 20 mg/kg/day, or placebo for 2 years. Patients were monitored by 2-weekly HU dosing weeks. Primary endpoints were preserved measured by 99mTc spleen scan and 99mTc renal clearance (GFR). Secondary endpoints included Hb and Hf; transcranial Doppler ultrasonography (TCD) and neurodevelopment (Bayley); growth; quantitative spleen function measured by spleen:liver counts, pitted cell counts, and Howell-Jolly bodies (HJB); DNA mutations; and clinical events.

Results: 193 subjects from 14 institutions were enrolled and 167 (86%) completed the study. Mean age was 13.6 months, 56% were female, and 96% had HbSS. The primary endpoint goals were not achieved, but markedly reduced VOE and improved hematologic counts were seen (Table). No toxicities except modest transient neutropenia occurred.

Conclusion: In these infants/toddlers with SCA, there was no clear impact of HU on primary spleen and renal endpoints, although positive effects were seen on spleen: liver count ratios, pit cells, HJB and urine osmolality. HU reduced complications (pain, dactylitis, ACS, transfusions, hospitalizations) and resulted in higher Hb, MCV, and HbF levels. HU, administered at a moderate dose, has substantial clinical benefit without serious toxicity in these very young children.

Table/Charts:

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RESULTS: Analysis was performed with all patients now at least 4 years from study entry. Patients treated with cohort 5 chemotherapy plus imatinib (N = 25) had a 3-year EFS of 84.7% (p = 0.93), which was not significantly different from all BMT cohorts (1-5) receiving related donor BM (64 ± 11%; N = 20), or unrelated, off protocol BM (63 ± 13%; N = 12, p = 0.73). A similar conclusion was reached when restricted only to cohort 5 BM patients that received continuous imatinib either before or after BM. Those updated data support the conclusion that patients with Ph+ ALL should be considered for treatment with imatinib plus intensive chemotherapy, rather than allogeneic BMT, in first remission.

Design/Method: Imatinib (340 mg/m²/day) with an intensive chemotherapy regimen was given on COG AALL0331 for children and adolescents (1-21 years) with Ph+ ALL (N = 92). Total exposure to imatinib was increased progressively in five patient cohorts with the best outcome in Cohort 5 (N = 50); with 658 continuous days of imatinib starting after conclusion of Induction therapy. Patients with HLA-identical sibling donors in cohort 5 underwent BM with imatinib given with chemotherapy for at least 42 days exposure before BM and for 180 days following BM.

Objectives: To critically evaluate the inclusion of signaling domains from leukemias refractory to traditional chemotherapy.

Design/Method: T lymphocytes gene modified to express anti-CD19-targeting CAR with TcRzeta alone, or a subset of MRD positive patients with a very good prognosis.

Conclusion: Longer follow-up continues to demonstrate that continuous imatinib with intensive chemotherapy provides an equivalent outcome to either related or unrelated donor allogeneic BM. This true even when the analysis is limited to cohort 5 patients who received continuous imatinib before and after BM. These results suggest that ALC, a simple test, constitutes a novel and powerful prognostic factor in pediatric ALL. ALC has prognostic significance that significantly adds to MRD-based risk stratification, notably with the ability to identify a subset of MRD positive patients with a very good prognosis.
on Day 7 (7 × 10^7 CAR +). Mice were followed weekly by quantitative flow cytometric analysis of peripheral blood for CD4, CD8, and CD19 positive human cells. Results: Mice treated with T cells engineered to express each of the anti-CD19 CAR demonstrated statistically significant improved survival over controls, with animals succumbing either to leukemia or, in a construct dependent manner, xenograft versus host disease (GVHD). The CD19-CD28-CD137-zeta CAR demonstrated the longest median overall survival and the most mice surviving until the end of the study at Day 180. In vivo bioluminescent imaging shows the kinetics of leukemia clearance to be rapid (within 72 hours), and proves a sensitive tool for tracking disease relapse. Conclusion: These data indicate it is possible to quickly eradicate established bone marrow disease with a single injection of lentiviral transduced CAR T cells. Future efforts will focus on enhancing in vivo persistence and surveillance capabilities of these CAR T cells, as well as investigating the ability of CAR T cells to clear repeated challenges of leukemia.

**Background:** Deletion of the IKZF1 gene encoding the lymphoid transcription factor IKAROS is an adverse prognostic factor in B-precursor ALL (1). We hypothesized that IKZF1 deletion may be directly linked to chemotherapy resistance and associated with disease recurrence in pediatric ALL.

**Objectives:** To determine the incidence of IKZF1 deletions in a panel of paired diagnosis/relapse ALL bone marrow samples and to determine if deletion alters patterns of chemosensitivity.

**Design/Method:** We investigated somatic copy number abnormalities in 76 diagnosis/relapse paired samples from children enrolled on COG protocols using Affymetrix Design/Method: diagnosis/relapse ALL bone marrow samples and to determine if deletion alters patterns of chemosensitivity.

**Results:** We detected IKZF1 deletions in 29 of 76 paired diagnosis/relapse samples. Of these, 22 pairs had a deletion at both diagnosis and relapse, whereas deletion was observed only at diagnosis or only at relapse in 2 and 5 cases, respectively. Twenty one deletions were focal (10 Mb). The percentage of cases with an IKZF1 deletion (38 %) was higher than previously reported for newly diagnosed ALL (8.9 % overall)(2). For chemoresistance studies, transfection efficiencies varied between 45–78% with a decrease in IKZF1 expression of 69-79%. The IC50 was significantly higher for etoposide (P = 0.009) and prednisolone (P = 0.03) in knock down samples relative to control but not for doxorubicin (P = 0.3). Conclusion: IKZF1 deletions were present at diagnosis in nearly 38% of patients who ultimately relapsed and IKZF1 knock-down led to chemoresistance to prednisolone and etoposide in ALL cell lines suggesting an essential role in mediating treatment failures in childhood ALL.


**Background:** Diamond Blackfan anemia (DBA) is one of the rare inherited bone marrow failure syndromes, characterized by erythroid hypoplasia, congenital anomalies and cancer predisposition. At least 50% of DBA patients have a ribosomal protein mutation, suggesting that DBA is a disorder of ribosome synthesis or function. Anemia is the most prominent feature of DBA, but the ontology and basis of the hematopoietic defect are unclear.

**Objectives:** Our goal was to analyze hematopoietic differentiation in Rps19 and Rpl5 mutant mouse ES cells in order to characterize the defect at the major stages of hematopoiesis.

**Design/Method:** We studied primary and secondary in vitro differentiation of murine mouse ES cells by inhibiting the destruction of misfolded proteins and inducing proteotoxicity. Current therapeutic strategies for hemophilia patients with inhibitors revolve around inducing tolerance to FVIII by treating patients with large doses of FVIII daily. This therapy induces antibody production, stressing the plasma cells that manufacture the inhibitor antibodies. Results: Bortezomib was shown to induce ER stress and apoptosis. The induction of ER stress was shown to require bortezomib induced oxidative stress. Induction of apoptosis was shown to involve the apoptotic mediators of the UPR. We demonstrate that stimulated B-lymphocytes maturing to plasma cells are more sensitive to mutant mouse inhibitor than unstimulated B-lymphocytes and that proteasome inhibitors decreased antibody secretion dramatically. Further, we show that GADDO4 and CHOP, mediators of the UPR, are needed to induce immune tolerance in our mouse inhibitor model.

**Conclusion:** The one-two punch of ER stress directed at the anti-FVIII plasma cells and proteasome inhibitor is an effective immune tolerance strategy.
Background: Children with sickle cell disease (SCD) have a markedly increased risk of invasive pneumococcal disease (IPD). In 2000, a heptavalent protein-conjugate pneumococcal vaccine (PCV7) was licensed. PCV7 was quickly adopted by the SCD community as an addition to prophylactic penicillin and the pneumococcal polysaccharide vaccine for prevention of IPD. Prior studies of PCV7's effectiveness have been limited in geographic scope and duration of follow-up.

Objectives: We sought to describe the national effectiveness of PCV7 in IPD prevention.

Design/Method: We analyzed the 1994-2007 National Inpatient Sample discharge databases which constitute a 20% stratified sample of all US hospitalizations and are weighted to allow national estimation. We identified discharges under age 18 with SCD by ICD-9-CM code. Within these, we identified IPD using ICD-9-CM codes 038.2 (pneumococcal sepsis), 038.1 (pneumococcal meningitis), and 041.2 (unspecified pneumococcal infection).

Results: For the 1243 IPD discharges from 1994-2007 the median age was 3 years; mortality was 2.4%. In the 7 years before and including PCV7 licensure (1994-2000), the mean annual IPD hospitalization rate in the US was 131.9 (95% Confidence Interval: 99.7-164.0; Figure). In the 7 years (2001-2007) that followed PCV7 licensure, the mean rate decreased to 45.7 (95% CI: 28.6-62.9; Figure). The mean proportion of IPD to total SCD hospitalizations decreased from 0.4 IPD cases / SCD discharge / year to 0.15 following licensure of PCV7 (p < 0.0001).

Conclusion: We show that national hospitalization rates for IPD have decreased nearly 3-fold since PCV7 licensure.
diagnosis and relapse sample. However, distinct gene expression signatures were identified for early and late relapse. Early relapse was characterized by 169 genes and late relapse was characterized by 192 genes, relative to diagnosis; only 14 genes were common to both. Pathway analysis of up-regulated genes at relapse revealed that similar pathways were shared between early and late relapse (mitosis, cell division, cell cycle), but the individual genes within these pathways differed; only 5 genes were commonly differentially expressed. Interestingly, several genes involved in nucleotide biosynthesis, which are the targets for antifolate therapy, were up-regulated selectively at late relapse, e.g., TYMS, CAD and IMPA2. Up-regulation of TYMS and IMPA2 in late relapse was validated using RT-PCR.

**Conclusion:** Gene expression profiling reveals unique gene signatures for early and late relapsed childhood ALL. Although there was overlap in the differentially expressed genes, a distinct subset of genes characterized late disease recurrence. Genes involved in nucleotide biosynthesis and folate metabolism were selectively up-regulated in late relapse and may be targets for therapy augmentation.

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**Background:** The CALM (Clathrin Assembly Lymphoid Myeloid leukemia) protein is involved in the formation of clathrin-coated vesicles that mediate the internalization of growth factor receptors; this process impacts the signaling and membrane expression of these receptors. Chromosomal translocations of CALM leading to its fusion with the AF10 gene are frequent in childhood and adult T-lineage leukemias. Retroviral transduction of CALM-AF10 immortalizes primary bone marrow cells and induces leukemia following transplantation into irradiated mice. The CALM-AF10 fusion protein conserves the majority of the CALM coding sequence, including a region mapping to amino acids 583-660 that mediates binding to clathrin.

**Objectives:** To study CALM’s ability to regulate the expression of growth factor receptors and determine whether this contributes to the oncogenic properties of CALM-AF10.

**Methods:** To assess the role of CALM we used fit1 mice that lack functional CALM protein expression. We created a CALM-/-AF10 mutant in which we deleted the CALM clathrin-binding domain (aa583-660) that we have previously shown to be important for binding clathrin. We compared the oncogenic properties of CALM-583, AF10 to that of CALM-AF10 using bone marrow retroviral transduction.

**Results:** We established cell lines from calms/- fit1 myeloid progenitors and found that CALM deficiency led to higher cell surface expression of the stem cell receptor (Kit) and the transferrin receptor. This suggests that CALM is important for regulating growth factor expression in hematopoietic cells. Whereas CALM-AF10 transplanted mice all developed leukemias within 3 months; a majority of CALM-583, AF10 mice survived more than 6 months. Interestingly, detection of CALM-583, AF10 transduced leukocytes by flow cytometric measurement of the green fluorescent protein (GFP)-marker in the blood of these mice showed a steady increase of these cells in the reconstituted mice over time, but paradoxically this was not accompanied by leukocytosis. This shows that CALM-583, AF10 confers a growth advantage to repopulating hematopoietic precursors but is a weaker oncogene than full length CALM-AF10.

**Conclusion:** Our finding that the clathrin-binding domain of CALM is required for the full leukemogenic potential of CALM-AF10 supports the hypothesis that deregulation of endocytosis and alteration of growth factor receptor expression both contribute to leukemogenesis.

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**Background:** The stem cell niche provides a micro-environment for stem cell signaling and as such offers a unique target for the development of novel stem cell therapies. Hematopoietic stem cells within the niche attach to osteoblasts and/or vascular sinusoid endothelial cells. We have recently identified mesenchymal stem cells (MSC) as niche-maker cells and found a crucial role of the SDF-1/CXCR4 axis in this process. Stromal Derived Factor-1 (SDF-1) regulates stem cell trafficking and the cell cycle via its receptor CXCR4. The correlation between leukemia stem cell biology and their niche is only emerging in pediatric leukemia studies.

**Objectives:** (1) To determine whether MSC can establish an ectopic human BM environment in NOD/SCID mice (2) To determine whether an ectopic human BM environment can create a leukemic niche for drug screening of niche-disrupting agents. (3) To determine whether the niche assay can be used to study homing and migration of human AML in vivo.

**Methods:** MSC-coated polyurethane scaffolds (Biomerix) were implanted subcutaneously in non-irradiated NOD/SCID mice (n=30). Normal CD34+ or primary AML cells (leukapheresis) were injected into the scaffold and analyzed for engraftment. The scaffolds were treated with SDF-1, AMD3100 (a CXCR4 antagonist) or PBS (control). After 4 weeks, AML survival in the mesenchymal niche was evaluated by immunohistochemistry. For homing studies, MSC-coated scaffolds were cut in half and each half was implanted at two different sides on the dorsum of the same mice. AML cells were injected at one side and homing of AML cells to the other side was analyzed at day 14.

**Results:** The MSC-coated scaffolds, in the presence of SDF-1 showed bloodvessels, osteoclasts and adipocytes present, suggestive of an ectopic human BM microenvironment in the mice. Quiescent AML cells (Ki67-) were shown to be “hinding” in the niche-sanity and the mesenchymal leukemic stem cell niche is regulated by the SDF-1/CXCR4 axis. AML cells seem to preferentially migrate and home to the ectopic human BM environment as the murine bone marrow remains clear of human AML.

**Conclusion:** The utilization of this assay will hasten the development of drugs for targeted-niche disruption in childhood AML and can improve therapeutic outcome.

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**Background:** Patient brain tumors cultured in the presence of growth factors can form multi-passageable neuropheres (NS) harboring tumorigenic cells with self-renewal and aberrant differentiation properties. Renewable neurosphere formation has been shown to be a strong independent predictor of clinical outcome in adult malignant gliomas, yet its prognostic role for pediatric brain tumors is unknown.

**Objectives:** We sought to determine whether formation of NS cultured from pediatric brain tumors is clinically relevant and reflective of clinical aggressiveness.

**Methods:** Established in vitro neurosphere conditions were used for culturing samples from glial, embryonal and mixed tumors from 56 pediatric patients. Potential associations between NS formation and clinical outcome were analyzed retrospectively.

**Results:** Thirty-seven percent of all samples formed NS. Three-year progression-free survival (PFS) was 71% ± 7% (mean ± std. error) and 3-year overall survival (OS) was 81% ± 6% in the full population. Analysis of available clinical outcome data from 51 patients demonstrated significantly increased hazard ratios (HR) for both disease progression (HR = 9.9, p < 0.001) and death (HR = 16.6, p < 0.01) in patients from whose tumors NS could be derived. The NS group had 33 ± 13% 3-year PFS and 50 ± 15% OS, whereas the group without NS had 86 ± 7.8% 3-year PFS and 97 ± 3.4% OS. Furthermore, the formation of NS correlated with adverse PFS in both subpopulations of glial and embryonal tumors, but not in mixed glial neuronal tumors. Overall survival was significantly worse for patients with tumors forming NS from embryonal and medulloblastoma subpopulations, but not in the glial group. Multivariate analysis showed that the presence of NS was associated with diminished PFS and OS independent of age, gender, or treatment. NS was an independent predictor of diminished PFS of glial tumors after adjusting for grade, as well. Moreover, multivariate analysis in the full population, adjusting for both K67 and NS formation, demonstrated that neurosphere formation remained predictive of progression whereas Ki67 did not.

**Conclusion:** The formation of NS derived from pediatric glial and embryonal brain tumors is more predictive of clinical progression than Ki67, reflects the biological aggressiveness of these tumors, and may provide a robust model for preclinical evaluation of therapeutics.

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**Background:** The ectopic human mesenchymal stem cell-coated scaffolds create an in vivo leukemia niche in NOD/SCID mice.

**Objectives:** To study the formation of mesenchymal stem cell sponge in vivo.

**Methods:** 1. To determine whether MSC can establish an ectopic human BM environment in NOD/SCID mice (2) To determine whether an ectopic human BM environment can create a leukemic niche for drug screening of niche-disrupting agents. (3) To determine whether the niche assay can be used to study homing and migration of human AML in vivo.

**Results:** The MSC-coated scaffolds, in the presence of SDF-1 showed bloodvessels, osteoclasts and adipocytes present, suggestive of an ectopic human BM microenvironment in the mice. Quiescent AML cells (Ki67-) were shown to be “hinding” in the niche-sanity and the mesenchymal leukemic stem cell niche is regulated by the SDF-1/CXCR4 axis. AML cells seem to preferentially migrate and home to the ectopic human BM environment as the murine bone marrow remains clear of human AML.

**Conclusion:** The utilization of this assay will hasten the development of drugs for targeted-niche disruption in childhood AML and can improve therapeutic outcome.
**SAFETY AND EFFICACY OF TANDEM (131I-METAIODOBENZYLGLUANIDINE INFUSIONS IN RELAPSED/REFRACTORY NEUROBLASTOMA**

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Background: Targeted radiotherapy with 131I-Metaiodobenzylguanidine (131I-MIBG) is safe and effective therapy for refractory neuroblastoma, with response rates greater than 30 percent. The primary toxicity is reversible myelosuppression.

**Objectives:** To determine the response rate for an early second (less than 100 days) 131I-MIBG treatment following an effective (stable disease or better) initial 131I-MIBG treatment.

**Design/Method:** Patients received an initial infusion of 131I-MIBG at activity level of 18 mCi/kg. Patients without progressive disease and available hematopoietic stem cell product were eligible for additional 18 mCi/kg 131I-MIBG therapies. Unresolved grade 3/4 thrombocytopenia did not preclude patients from receiving multiple infusions. Subsequent treatments were administered a minimum of six weeks and a maximum of 100 days from previous MIBG therapy.

**Results:** Seventy-six subjects with refractory neuroblastoma were treated with 18 mCi/kg 131I-MIBG. Patients were heavily pretreated, with a median number of 4 prior chemotherapy regimens (1-8), and many also had received prior radiation therapy (76.5%), prior myeloablative therapy (73.7%), and/or prior biologic therapy (75.9%). Activity against neuroblastoma was measured at 6 weeks and showed 6.6% CR, 23.7% PR, 48.7% SD and 21.4% PD. Forty one patients received a second 18 mCi/kg dose a median of 59 (46-93) days following the first infusion. Response among the patients receiving tandem infusions showed 12.2% CR, 17.1% PR, 36.6% SD and 34.1% PD. Four of 5 initial CRs were maintained, despite all 5 showing positive immediate post-treatment 131I-MIBG scans after the second infusion. Better response to the second infusion compared to the first was seen in 12.5% of subjects. After two treatments, 16 of 41 patients (39%) experienced a reduction in overall disease burden. An additional 6 had evidence of improvement by MIBG scan. The therapy was well-tolerated, with 35 patients receiving PBSC support after the second treatment (median dose of 18 mCi/kg) after second infusion.

**Conclusion:** An early second 131I-MIBG infusion results in an improved overall response rate following an effective initial infusion. The continued effectiveness of 131I-MIBG after consecutive infusions in rapid succession suggests that this strategy should be incorporated into therapy of refractory neuroblastoma.

**THE POTENTIAL ROLE FOR GENOTYPES OF KILLER IMMUNOGLOBULIN-LIKE RECEPTORS, THEIR LIGANDS, AND FC RECEPTORS TO IGG ON ANTITUMOR RESPONSES OF NEUROBLASTOMA PATIENTS RECEIVING THE Hu14.18-IL2 IMMUNOCYTOKINE: A CHILDREN'S ONCOLOGY GROUP REPORT**


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Background: Response to immunocytokine (IC) therapy is dependent on natural killer (NK) cells in murine neuroblastoma (NBL) models. Furthermore, killer immunoglobulin-like (KIR) receptor-ligand mismatch is associated with improved outcome in haploidentical transplantation for leukemia and autologous transplant for NBL. Additionally, anti-tumor response to monoclonal antibodies has been associated with specific polymorphic FcγR alleles. We show that FGFR4 is a downstream target of PAX3-FOXO1 and that loss of function of FGFR4 inhibits aRMS cell growth and tumorigenesis, we used shRNA knockdown of FGFR4 expression. To pharmacologically inhibit FGFR signaling, we used the FGFR small molecule inhibitor PD173074. Studies were performed in human skeletal muscle myoblasts, human RMS cell lines, and subcutaneous murine xenografts.

**Results:** We show that FGFR4 is a downstream target of PAX3-FOXO1 and that loss of function of FGFR4 inhibits aRMS cell growth by inducing apoptosis. This apoptotic induction is reduced with expression of BCL2L1, suggesting that FGFR4 regulates anti-apoptotic signaling pathways in aRMS. In eRMS cell lines, knockdown of FGFR4 inhibits eRMS tumor cell growth in vitro and in vivo in murine xenografts by inhibiting proliferation. The FGFR small molecule inhibitor PD173074 inhibits both eRMS and aRMS cell growth in vitro, and induces aRMS tumor cell apoptosis in vivo.

**Conclusion:** FGFR4 has divergent roles in eRMS and aRMS, to promote cell proliferation or inhibit apoptosis, respectively. Our studies show that FGFR4 regulates critical signaling pathways in RMS and define FGFR4 as a new therapeutic target for RMS.

**SAFETY, DOSE ESCALATION AND EFFICACY OF GENITUZUMAB OZOGAMICIN IN COMBINATION WITH A MYEOABLATIVE CONDITIONING REGIMEN AND ALLOSC IN CHILDREN WITH HIGH-RISK CD33+ AML AND MYELOID/PLASMYND SYNDROME: A NOVEL CHEMOMMUNOTHERAPY CONDITIONING REGIMEN**


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Background: High-risk AML (induction failure [IF], refractory relapse [RR], 3rd complete remission [CR3]) has dismal outcomes (OS 10-20%) (Shenoy, et al, 2010).
Followed by AlloSCT. GVHD prophylaxis consisted of tacrolimus/mycophenolate doses of 3.0, 4.5, 6.0, or 7.5 mg/m². No dose-limiting toxicities secondary to GO were observed.

Seven patients enrolled, median age 10 yrs (range 6–18), 5/2 M/F, 5 ALL (3 CR3, 1 CR, 1 relapse). 2 ALL (1 treatment-related AML in CR3, 1 IF), 5/2 related/untreated donors, median total matched cell dose 2.28–8.90. CD34 dose 5.03 × 10^9/kg (2.41-7.02). An MTD of clofarabine has yet to be reached: no serious adverse events related to clofarabine have been observed. All engrafted neutrophils at median day 14 (12-27). All achieved 100% donor chimerism by day 42 (12-40); 75% of patients engrafted platelets at median day 42 (21-164). Median day +30 donor chimerism was 99% (85-100%). The probability of grade II-IV aGVHD was 42% of GVHD was 28%. Four of 12 patients died from progressive disease. Overall disease-free survival during the entire follow-up period was 50% (95% CI: 20.8-73.6%); median survival time was 471 days (206-2055).

Conclusion: Incorporation of GO combined with a MAC regimen (Bu/Cy) followed by AlloSCT is well-tolerated in children with high-risk MDS/AML and is associated with improved EFS and OS compared to historical controls. The MTD of GO plus Bu/Cy has yet to be determined.

(PAPER 101)
LEUKEMIA ANTIBODIES IN SURVIVORS OF ALLOGENEIC TRANSPLANT: POTENTIAL ROLE OF B CELLS IN GVL EFFECTS

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Background: Allogeneic hematopoietic stem cell transplantation is performed for high risk acute lymphoblastic leukemia (ALL). Complex allogeneic immune responses can produce graft versus host disease which is associated with antileukemia effects. The prevailing paradigm is that these are mediated by allogeneic T cells. Our laboratory has been studying the effects of post transplant vaccination and donor lymphocyte infusions in murine models of allogeneic transplantation.

Objectives: Our goal was to identify in transplant hosts treated with DLI and leukemia vaccines immune responses that may have contributed to long term control of ALL. Our hypothesis was that in survivors we would find robust donor T cell responses to allogeneic minor histocompatibility antigens and to leukemia restricted antigens

Design/Method: Using a well characterized murine model of MHC-matched, multiple minor histocompatibility mismatched transplantation mice were challenged with malemurine pre-B ALL driven by a bcr/abl p210 oncogene and a Lck/Arf deletion, lesions frequently present in ALL. Some were treated with DLI and/or leukemia vaccines comprised of irradiated leukemia cells, Freund’s adjuvant and GM-CSF. After two months immune responses were measured. T cell responses were measured with ELISPOT assays specific for minor histocompatibility leukemia specific responses. Antibody responses were measured by flow cytometry and ELISA. Leukemia was measured by flow cytometry.

Results: Hosts treated with DLI and vaccines exhibited reduced leukemia burden two weeks after challenge. In survival experiments untreated mice had 100% mortality, while 20% of treated mice were survivors. Contrary to our hypothesis robust T cell responses were not present in survivors. Surprisingly leukemia reactive IgM and IgG antibodies were found in titers of 1:80. These antibodies did not bind nonmalignant donor or recipient marrow cells, but did react with a number of independently derived C57BL/6 leukemia cell lines.

Conclusion: Antibody responses to ALL cells are consistently observed in survivors. These studies do not prove the antibodies play a role in control of leukemia, but suggest the hypothesis that antibody responses may contribute to GVL effects. Current experiments in B cell deficient mice are underway to test the mechanistic role of antibody responses in the GVL effect.

(PAPER 102) PRELIMINARY RESULTS OF DOSE ESCALATION OF CLOFARABINE IN COMBINATION WITH CYTARABINE AND TOTAL BODY IRRADIATION (TBI) FOLLOWED BY ALLOGENEIC STEM CELL TRANSPLANTATION (ALLOSCRT) IN CHILDREN AND YOUTH: ADULTS (CAYA) WITH POOR-RISK ACUTE LEUKEMIA

Mark Geyer, Lauren Harrison, Nathifa Fearon, Claire Handler, Deirdre Duffy, Monica Bhatia, James Garvin, Diane George, Prakash Satwani, Alexandra Cheever, Julie Talano, Joseph Schwartz, Lee Ann Baxter-Lowe, Mitchell Cairo, Morgan Stanley Children’s Hospital of New York-Presbyterian, Columbia University, New York, NY, United States

Background: The prognosis is poor for CAYA with ALL or AML in CR3 or with relapsed or refractory disease. Single-agent clofarabine has demonstrated activity in pediatric patients with relapsed/refractory ALL and AML. (Jeha et al., Blood, 2004 and JCO, 2006); reports have demonstrated increased overall response rates with combination therapy using clofarabine, cyclophosphamide and etoposide (Hijiya et al., Leukemia, 2009). Additionally, biochemical and clinical studies suggest synergy with sequential cytarabine (Faderl et al., Blood, 2006).

Objectives: To determine the maximum tolerated dose (MTD) of clofarabine in combination with cytarabine and TBI followed by AlloSCT and to assess the safety, disease-free survival, and overall survival associated with this conditioning regimen in CAYA with poor-risk acute leukemia.

Design/Method: CAYA with ALL or AML in CR3, relapse, or induction failure (IF) received 5 days (day-9 through day-5) of clofarabine (dose escalation with 40mg/m² [3], 46 mg/m² [3], 52mg/m² [1]) and sequential cytarabine 1000mg/m² (cytarabine also administered day-10). TBI (120GyGy) + R-ATG (unrelated donors only) followed by AlloSCT from matched related or unrelated donors. GVHD prophylaxis consisted of tacrolimus and MMF as we have recently described (Bhatia/Cairo et al., BBMT, 2009).

Results: Seven patients enrolled, median age 10 yrs (range 6–18), 5/2 M/F, 5 ALL (3 CR3, 1 CR, 1 relapse). 2 AML (1 treatment-related AML in CR3, 1 IF), 5/2 related/untreated donors. Seven patients at median day 22 (12-40); 75% of patients engrafted platelets at median day 42 (21-164). Median day +30 donor chimerism was 99% (85-100%). The probability of grade II-IV aGVHD was 42% of GVHD was 28%. Four of 12 patients died from progressive disease. Overall disease-free survival during the entire follow-up period was 50% (95% CI: 20.8-73.6%); median survival time was 471 days (206-2055).

Conclusion: Incorporation of GO combined with a MAC regimen (Bu/Cy) followed by AlloSCT is well-tolerated in children with high-risk MDS/AML and is associated with improved EFS and OS compared to historical controls. The MTD of GO plus Bu/Cy has yet to be determined.

Research supported by Genzyme.

(PAPER 103) ANALYSIS OF THE INCIDENCE AND CHARACTERISTICS OF VENOOCCLUSIVE DISEASE (VOD) OF THE LIVER IN PEDIATRIC HEMATOPOIETIC PROGENITOR CELL TRANSPLANT (HPCT) PATIENTS WITH HEPARIN ALONE VS. HEPARIN AND URSODIOL PROPHYLAXIS AT THE MEDICAL COLLEGE OF WISCONSIN

Jessica Brown, Julie Talano
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Background: VOD, a serious and potentially life-threatening complication of HPCT, results from liver injury due to chemotherapy and/or radiation. The reported incidence rate in pediatric HPCT patients varied from 5% to 40%. Previous studies show the beneficial effects of post-transplant pharmacological therapies such as ursodeoxycholic acid (ursodiol), heparin, and dextrose at preventing VOD. However, the combined effect of heparin and ursodiol prophylaxis in preventing VOD in pediatric patients has yet to be determined.

Objectives: Evaluate the effect of heparin alone or combined with ursodiol/anticlotting/TBI on the incidence of VOD in pediatric HPCT patients.

Methods: A retrospective chart review was performed to evaluate the characteristics and incidence of VOD in patients who underwent transplantation from 1996-2002, who received heparin alone compared to those patients in 2003-2008 who received the combination of heparin and ursodiol. Patients were identified through medical records with the ICD diagnosis of VOD. We performed a retrospective chart review to evaluate the characteristics and incidence of VOD in patients who underwent transplantation from 1996-2002, who received heparin alone compared to those patients in 2003-2008 who received the combination of heparin and ursodiol. Patients were identified through medical records with the ICD diagnosis of VOD.

Results: The day 100 survival in the VOD patients was 70% while 75% of patients engrafted platelets at median day 42 (21-164). Median day +30 donor chimerism was 99% (85-100%). The probability of grade II-IV aGVHD was 42% of GVHD was 28%. Four of 12 patients died from progressive disease. Overall disease-free survival during the entire follow-up period was 50% (95% CI: 20.8-73.6%); median survival time was 471 days (206-2055).

Conclusion: The incidence of VOD is lower in children receiving heparin and ursodiol prophylaxis compared to those patients receiving heparin alone. The incidence of VOD is lower in children receiving heparin and ursodiol prophylaxis compared to those patients receiving heparin alone. The incidence of VOD is lower in children receiving heparin and ursodiol prophylaxis compared to those patients receiving heparin alone. The incidence of VOD is lower in children receiving heparin and ursodiol prophylaxis compared to those patients receiving heparin alone. The incidence of VOD is lower in children receiving heparin and ursodiol prophylaxis compared to those patients receiving heparin alone.
Conclusion: Low dose heparin and ursodiol prophylaxis appears to be an effective strategy, more so than heparin alone, in VOD prevention in pediatric patients. Future prospective trials are needed in pediatric HPC patients to further elucidate the most efficacious and cost effective VOD prophylaxis, since currently there is no consensus of what constitutes standard of care among pediatric HPC centers.

### Tables/Charts:

**Table 1: Group I: Hepatotoxicity (n=10) vs Group II: Hepatotoxicity + Ursodiol (n=20)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Hepatotoxicity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>60 (60.0%)</td>
</tr>
<tr>
<td>II</td>
<td>40 (60.0%)</td>
</tr>
</tbody>
</table>

### References:

1. Lobrich, PNAS, 2005
2. Seibel et al. 2005
3. Obriet al demonstrated that computed tomography (CT) induced double-stranded DNA breaks were quantifiable by increased γH2AX foci in lymphocytes. The number correlated linearly to radiation dose and returned to baseline by 24 hours in all but one patient. In this patient, who had suffered unanticipated, severe side effects from previous radiation therapy, foci were increased at all time points and remained elevated at 24 hours.

### Objectives:

Our objective was to measure γH2AX foci induced by a pre-transplantation diagnostic CT in patients with FA as a potential marker of individual sensitivity to radiation and chemotherapy used in transplantation preparative regimens.

### Design/Method:

Six patients with FA were enrolled and blood samples were obtained prior to radiation exposure and at 30 minutes, 60 minutes and 24 hours post scan. Fixed lymphocytes were stained with anti-γH2AX, and foci were enumerated using fluorescence microscopy. Clinical phenotype and adverse effects during transplantation were recorded.

### Results:

γH2AX foci were increased in all FA patients (n=6) after diagnostic CT scan. The number of foci per cell at 30 minutes (normalized for radiation dose) varied two-fold among FA patients (range 0.19–0.39). Foci decreased at 60 minutes (range 0.10–0.26) and returned to baseline at 24 hours (range 0.01–0.03) in all subjects.

### Conclusion:

The decrease in foci seen at 60 minutes and return to baseline at 24 hours suggests, perhaps surprisingly, prompt and effective DNA repair of radiation induced DNA injury in patients with Fanconi anemia. It remains unclear in this small cohort whether this assay predicts sensitivity. Additional children with FA, as well as normal pediatric controls, are being recruited to answer the question whether the CT induced foci correlate with severity of malformation phenotype and predict clinically important variations in tolerability of therapy.

(Lobrich, PNAS, 2005)
(POSTER 107) ADVERSE OUTCOMES IN UNDERWEIGHT CHILD AND ADOLESCENT SURVIVORS OF CANCER AND RECIPIENTS OF HEMATOPOIETIC STEM CELL TRANSPLANTS

Jamie Dargart, Kimberly Dilley
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Background: Results from the Childhood Cancer Survivors Study have shown that childhood cancer survivors are more likely to be underweight compared to their siblings and the general population. These underweight survivors are more likely to experience adverse health outcomes.

Objectives: The objective of this study was to compare underweight child and adolescent survivors of cancer and recipients of hematopoietic stem cell transplants to age- and diagnosis-matched controls.

Design/Method: Patients eligible for retrospective medical record review were seen in a single institution long-term survivor clinic. Recent weight and height measurements were used to determine weight status according to CDC guidelines. Non-underweight controls were matched to underweight cases based on diagnosis category and age at diagnosis, then gender and race/ethnicity, when possible. McNemar test or paired Student’s t-test was used for comparisons, as appropriate.

Results: 33 case-control pairs were identified. Acute lymphoblastic leukemia was the most common diagnosis, followed by Wilms tumor, neuroblastoma, and rhabdomyosarcoma. The number of pairs mismatched for diagnosis, gender, race/ethnicity, treatment, or time to follow up was not significant. Adverse outcomes by category are shown in Table 1.

Conclusion: Most case and control subjects experienced adverse outcomes within 10 years. There was a trend toward an increased prevalence of most adverse outcomes among underweight survivors, with the exception of metabolic disorders. Further prospective studies are needed to determine the causal relationship between weight status and the risk of developing adverse health outcomes.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Adverse Outcome</th>
<th>Cases n = 33 (%)</th>
<th>Controls n = 33 (%)</th>
<th>McNemar p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asthenia</td>
<td>10 (30.3)</td>
<td>6 (18.2)</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>Cardiac</td>
<td>7 (21.2)</td>
<td>3 (9.1)</td>
<td>0.344</td>
<td></td>
</tr>
<tr>
<td>Dental</td>
<td>4 (12.1)</td>
<td>3 (9.1)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Dermatologic</td>
<td>6 (18.2)</td>
<td>4 (12.1)</td>
<td>0.687</td>
<td></td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>10 (30.3)</td>
<td>10 (30.3)</td>
<td>0.190</td>
<td></td>
</tr>
<tr>
<td>Ocular failure</td>
<td>0 (0.0)</td>
<td>4 (12.1)</td>
<td>0.399</td>
<td></td>
</tr>
<tr>
<td>Ocular failure (n = 12)</td>
<td>2 (6.1)</td>
<td>2 (6.1)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Ocular failure (n = 13)</td>
<td>6 (18.2)</td>
<td>1 (3.0)</td>
<td>0.063</td>
<td></td>
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<tr>
<td>Metastatic</td>
<td>12 (36.4)</td>
<td>12 (36.4)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Growth-hormone deficiency</td>
<td>9 (27.3)</td>
<td>4 (12.1)</td>
<td>0.227</td>
<td></td>
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<tr>
<td>Hypothyroidism</td>
<td>5 (15.2)</td>
<td>3 (9.1)</td>
<td>0.727</td>
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<tr>
<td>Growth-hormone hormone</td>
<td>6 (18.2)</td>
<td>7 (21.2)</td>
<td>0.634</td>
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<tr>
<td>Hematologic Graft</td>
<td>2 (6.1)</td>
<td>2 (6.1)</td>
<td>1.000</td>
<td></td>
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<tr>
<td>Metacolestrol</td>
<td>5 (15.2)</td>
<td>6 (18.2)</td>
<td>0.471</td>
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<tr>
<td>Methotrexate</td>
<td>11 (33.3)</td>
<td>6 (18.2)</td>
<td>0.277</td>
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<tr>
<td>Methotrexate</td>
<td>13 (40.3)</td>
<td>13 (40.3)</td>
<td>0.727</td>
<td></td>
</tr>
<tr>
<td>Myelosuppression</td>
<td>2 (6.1)</td>
<td>2 (6.1)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>DVT/Venous</td>
<td>7 (21.2)</td>
<td>10 (30.3)</td>
<td>0.609</td>
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<tr>
<td>Bacterial superinfection</td>
<td>0 (0.0)</td>
<td>1 (3.0)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Pneumonia</td>
<td>0 (0.0)</td>
<td>3 (9.1)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Metabolic</td>
<td>4 (12.1)</td>
<td>3 (9.1)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Secondary malignancy</td>
<td>2 (6.1)</td>
<td>0 (0.0)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Any adverse outcome</td>
<td>16 (48.5)</td>
<td>7 (21.2)</td>
<td>0.057</td>
<td></td>
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</tbody>
</table>

(POSTER 108) THE ROLE OF PULMONARY FUNCTION TEST FOR THE EARLY DETECTION OF SUBSEQUENT LATE-ONSET NON-INFECTION PULMONARY COMPLICATIONS IN CHILDREN

Meerim Park, Keun Wook Bae, Kyung Nam Koh, Ho Joon Im, Jong Jin Seo, Hyung Nam Moon
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Background: Late-onset non-infectious pulmonary complication (LONIPC) is a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). But it is difficult to identify this disorder in the early stage because clinical symptoms of LONIPCs may be insidious and routine radiological examination may be normal.

Objectives: This study evaluated the incidence and the outcome of LONIPCs to identify risk factors and aimed to investigate whether changes in pulmonary function occur in the early stage of LONIPCs.

(POSTER 109) OUTCOME AFTER HEMATOPOIETIC STEM CELL TRANSPLANTATION IN CHILDREN WITH HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS AT THE HOSPITAL FOR SICK CHILDREN, TORONTO (SICKKIDS)

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The Hospital for Sick Children, Toronto, Ontario, Canada

Background: Hematopoietic stem cell transplantation (HSCT) is the only curative option for pediatric patients with primary hemophagocytic lymphohistiocytosis (HLH) as well as for patients with secondary HLH who fail to respond to therapy.

Objectives: To report outcomes and HSCT complication of pediatric patients with HLH.

Design/Method: A retrospective chart review of pediatric recipients of HSCT for HLH between June 1995 and Aug 2009 at SickKids Hospital. Event free survival was defined as no evidence of relapse, graft failure (GF) or death. Univariate analysis was utilized to assess factors influencing survival.

Results: Sixteen children (10 male), median age 1.2yrs (5m-16yrs) received a HSCT for HLH. Fourteen children had primary HLH. At the time of transplant, 2 children had active HLH and 2 were in partial remission. Median time to HSCT from diagnosis was 6 months (range: 3–39m). Donor sources were: sibling marrows-6, cord blood stem-cells-6, living unrelated stem-cells-5 and haplo-transplant-1. Thirteen children received fully matched donor stem-cells. Sixteen received myeloablative and 2 received reduced intensity conditioning.

Median time to neutrophil engraftment in 14 children was 16.5d (12-29d). Three patients died from multi-organ failure before day 100, and another patient died from pulmonary hemorrhage after day 100. Two children with primary GF developed recurrent HLH and died from complications after a second HSCT. One patient developed anaplastic large cell lymphoma. Three of 4 children not in complete remission at the time of transplantation died. Eleven children (61%) are alive at a median follow-up of 55.5m (4.5-122m). The following factors were not statistically associated with survival: acute GVHD, stem cell source, underlying disease or conditioning regimen.

Conclusion: Early mortality prior to day 100 was low (16%). Overall survival of 61% is similar to most published series. High infection rate and organ dysfunction complicate outcome. Reduce intensity conditioning may improve outcome after HSCT in HLH.

(POSTER 110) PRETRANSPLANT CONDITIONING WITH CAMPATH-III (ALEMTUZUMAB) IN PEDIATRIC MATCHED UNRELATED BONE MARROW TRANSPLANTS - AN INSTITUTIONAL EXPERIENCE

Amulya Nageswara Rao, Riten Kumar, Julia Gourde, Vilmarie Rodriguez, MD, Shakila Khan

2010 ASPHO ABSTRACTS 795
Background: Graft versus host disease (GVHD) remains a major cause of mortality and morbidity following matched unrelated hematopoietic stem cell transplantation (HSCT). Campath-1H (Alemtuzumab) is a humanized monoclonal antibody to CD52, an antigen expressed on T and B lymphocytes, monocytes and natural killer cells, and is thought to reduce GVHD incidence through in vivo T cell depletion. However, the same mechanism can potentially increase the risk of relapse and infection.

Objectives: To assess the outcome with Campath-1H, used in our pediatric matched unrelated donor pretransplant conditioning regimen, in relation to: GVHD, infections, relapse, and survival.

Design/Method: Retrospective case study and a literature review.

Results: The study included 17 pediatric patients (9 male; 8 female). Median age at transplant was 12.2 years (range: 0.7–19.7 years). 9 patients had leukemia/lymphoma, 3 had MDS/MDS evolving into AML and 5 had severe aplastic anemia. Pretransplant conditioning regimen was with Campath-1H given mainly with cyclophosphamide and total body irradiation. Campath-1H dosing was body weight based with 3 doses when treating a malignancy and 4 doses with bone marrow failure states. 16 patients engrafted (median time: 21 days). GVHD prophylaxis was mainly with tacrolimus/methotrexate. 5 developed Grade I-II acute GVHD. None developed chronic GVHD. One patient had a CMV reactivation but none developed active disease. 4 had varicella. 5 patients relapsed with 3 relapsing within +100 days. One had PTLD which was successfully treated with rituximab. 3 patients died due to relapse and one due to disseminated varicella infection. Median follow-up time was 719 days (range: 147–2175 days). Overall survival calculated using the Kaplan-Meir analysis was 100% and 94% at 100 days and 1 year respectively. Event free survival censoring for death, relapse and rejection was 76% and 64% at 100 days and 1 year respectively.

Conclusion: Our experience suggests Campath-1H effectively reduces the risk of serious GVHD, as previously reported, with no increase in serious infections. Given our small patient numbers the effect of primary disease on relapse rates could not be assessed. Larger studies comparing Campath-1H with conventional regimens are required to further assess relapse rates and to establish if the decreased GVHD incidence is sustained in larger cohorts.

(POSTER 112) SAFE AND EFFECTIVE REDUCED-COST BONE MARROW TRANSPLANTATION FOR THALASSEMA: THE PRELIMINARY EXPERIENCE OF THE CURE2CHILDREN FOUNDATION IN PAKISTAN.

Lawrence Faulkner, Saqib Ansari, Sadaf Khalid, Nalia Yaqub, Tasneem Farzana, Kamran Rashid, Yasir Iqbal, Pietro Sodani, Cristiano Gallucci, Buket Erer, Tahir Shamsi

Cure2Children Foundation, Florence, Florence, Italy

Background: Cure2Children Foundation, Florence, Italy; National Institute of Blood Diseases (NIBD), Karachi, Pakistan; Pakistan Institute of Medical Sciences (PIMS), Islamabad, Pakistan; Shifa International Hospital (SIH), Islamabad, Pakistan; Mediterranean Institute of Hematology, Rome, Italy.

The Cure2Children Foundation has supported both financially and professionally a network of centers in Pakistan performing bone marrow transplantation (BMT) for the cure of transfusion-dependent thalassemia.

Objectives: To evaluate safety, efficacy and cost-effectiveness of matched-related BMT for a homogeneous group of low-risk children with thalassemia major as performed by a network of institutions supported by a non-profit organization in a low-resource setting.

Design/Method: Matched-related bone marrow was administered after conditioning with thiotepa 10 mg/kg, busulfan 14 mg/kg and cyclophosphamide 200 mg/kg, followed by GVHD/rejection prophylaxis with prednisone, methotrexate, and cyclosporin. Management standards for therapy administration, central venous access, severe pancytopenia, immunosuppression, and hospital infection control have been addressed by local training, web-based data management and videoconferencing.

Results: Since August 2009, a total of 16 BMTs have been performed in low-risk patients with a median age of 3.3 years (range 0.9 to 6.2, 7 males and 9 females), and liver < 2cm. Nine BMTs were performed at an established center (NIBD), and 7 at newly developed services (5 at PIMS and 2 at SIH).

At a median follow up of 248 days (range 12–492), actuarial thalassemia-free survival is 87% and overall survival 100%. So far 2 patients had a graft failure and are alive and well after autologous reconstitution. One patient developed grade 3 and one grade 2 acute GVHD, 5 had subclinical CMV activation by RT-PCR and one developed pulmonary tuberculosis. Other manageable complications included hypertension and hemoglobin cystitis. No case of VOD or chronic GVHD has been observed.

The cost of setting up a two-bedded BMT unit (at PIMS) including civil works and basic equipment was 50,000 USD and that of each BMT was in the range of 20,000 USD, including full family support for a minimum of 8 months.

Conclusion: In low resource settings safe and effective bone marrow transplantation can be performed with a fraction of the costs compared to western centers.

(POSTER 113) DISEASE-SPECIFIC HEMATOPOIETIC CELL TRANSPLANTATION: REDUCED INTENSITY CONDITIONING REGIMEN FOR DYSKERATOSIS CONGENITA

Andrew Dietz, Paul Orchard, K. Scott Baker, Roger Giller, Sharon Savage, Blanche Alter, Jakub Tolar

University of Minnesota, Minneapolis, Minnesota, United States

Background: Dyskeratosis congenita (DC) is characterized by the clinical triad of reticular skin pigmentation, oral leukoplakia, and abnormal nails. Patients with DC have very short telomeres and about one-half have mutations in telomere biology genes. A majority of patients with DC develop bone marrow failure (BMF) refractory to immunosuppression. Hematopoietic cell transplantation (HCT) represents the only known cure for BMF in DC, but poses significant toxicities due to tissue injury from chemotherapy and radiation.

Objectives: We report six patients who underwent allogeneic HCT with a novel reduced intensity conditioning (RIC) regimen specifically designed for DC patients.

Design/Method: Graft sources included related peripheral blood stem cells (1), unrelated bone marrow (2), and unrelated double umbilical cord blood (3).

Results: Complete donor engraftment was achieved in 5 of 6 patients. One patient had initial autologous hematopoietic recovery, which was followed by a second transplant that resulted in 88% disease control and minimal costs and ethical concerns. With a median follow-up of 26.5 months, four patients are alive, three of whom were recipients of unrelated grafts.

Conclusion: Encouraging short-term survival can be achieved with HCT in patients with DC utilizing a preparative regimen designed to promote donor engraftment and minimize life-threatening disease-specific complications such as pulmonary fibrosis.
Hematopoietic differentiation defects in RPS19-deficient murine embryonic stem cells and rescue by gene replacement

Sharon Singh, Tracie Goldberg, Adrianna Henson, Abdallah Nihrane, Jeffrey Lipton, Steven Ellis, Johnson Liu

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Background: Diamond Blackfan anemia (DBA) is one of the rare inherited bone marrow failure syndromes (IBMFES), characterized by erythroid hypoplasia, congenital anomalies and a cancer predisposition. DBA is the first human disease caused by ribosomal protein haploinsufficiency, which somehow triggers apoptosis of erythroid precursors, possibly through activation of p53. The only therapies available for this disease are steroids, blood transfusions and bone marrow transplantation, each with significant associated morbidity. Therefore, new and targeted therapies with less toxicity are urgently needed.

Objectives: Our goal was to create a murine embryonic stem (ES) cell model of DBA with a mutation in Rps19 to study the pathophysiology of DBA and to test new therapies.

Design/Method: The Rps19-mutated murine embryonic stem cell lines, S17-10H1 and YHC074, were created using a gene trap strategy. The ES cells were induced to undergo primary differentiation into embryoid bodies (EBs). Day 9 EBs, representing definitive hematopoiesis, were re-plated with hematopoietic cytokines (SCF, IL-3, IL-6 and epo) in methylcellulose and secondary differentiation colonies were scored on day 10. Both mutant cell lines underwent electroporation with a plasmid vector expressing wild-type Rps19 or DNA.

Results: Western blot analyses confirmed S17-10H1 and YHC074 Rps19-haploinsufficiency, and polysome analysis showed reduction in the 40S subunit peak in YHC074 ES cells. Both cell lines had reduced EB formation (hematopoietic and non-hematopoietic) following primary differentiation. Significant defects in both erythroid (BFU-E) and myeloid (CFU-GM) formation were found following secondary hematopoietic differentiation of day 9 EBs. These defects were specific to Rps19 haploinsufficiency since all defects were rescued by stable transfection of the mutant cell lines with an Rps19-expressing correction vector.

Conclusions: Haploinsufficient defects in our murine ES cell models of DBA are specifically due to haploinsufficiency of Rps19. This system provides a powerful method for in vitro testing of novel therapeutics such as leucine, lenalidomide and p53 inhibition.

Zinc supplementation improves bone density in patients with thalassemia

Ellen Fung, Janet Kwiatkowski, James Huang, Ginny Gildengorin, Janet C. King, Elliott Vichinsky

Children’s Hospital & Research Center, Oakland, Oakland, CA, United States

Background: Previous reports have shown that up to 70% of patients with thalassemia have low bone mass despite current treatment regimens. Low bone mass in thalassemia (Thal) has been linked with low plasma zinc.

Objectives: To determine the effect of zinc supplementation on bone mass in young patients with Thal.

Design/Method: Forty-one subjects (21 Females; 10-30 years) with Thal were randomly assigned to receive 25 mg/d zinc or placebo. Subjects with 25-OH vitamin D (25OHD) levels <30 ng/dL were supplemented with vitamin D. Bone mineral density (BMD) of the spine, hip and whole body were assessed by dual energy x-ray absorptiometry and dietary intake by food-frequency questionnaire at 0, 12 and 18 months. BMD Z-scores were computed from manufacturer’s reference data. Fasting blood was collected for assessment of bone turnover markers at 0, 3, 6, 12 and 18 months.

Results: Thirty-three subjects completed the study (16.9±5.1 y, Mean±SD), 67% were B-Thal, 81% transfused. Zinc intake averaged 87.3±20.8 mg/d. Plasma zinc was 87.3±13.6 μg/dL. Plasma zinc was low in only 2 subjects, though 61% had 25OHD levels <30 ng/mL at baseline. Spine BMD Z-score averaged -2.0±0.8, hip Z= -1.3±0.9 and whole body Z= -1.9±1.1. Using intention to treat analysis, after 18 months the zinc group had significantly greater increases in hip BMD (3.8% increase over the control group, p=0.04), whole body BMD (4.1% increase, p=0.03) and BMD (2.3% increase, p=0.04). Osteocalcin levels mirrored the increases in the zinc group, p=0.01. In the sub-group with low 25OHD at baseline, zinc had a slightly greater effect on hip BMD (p=0.03). No significant effect of zinc supplementation was observed on BMD Z-scores, body composition, growth, or serum copper.

Conclusion: These results suggest that patients with Thal are functionally zinc depleted due to the positive effect of supplemental zinc on BMD. Plasma zinc is an unreliable indicator of status in Thal. This well-tolerated supplement may be beneficial for optimal bone health in both transfused and non-transfused Thal patients.

Safety and efficacy of zoledronic acid in treatment of low bone mineral density in patients with beta thalassemia major

Rahul Naithani, Tulika Seth, Nikhil Tandon, Jagdish Chandra, Harra Prasad Patti, Ved Prakash Choudhry, Reni Saxena

All India Institute of Medical Sciences, New Delhi, Delhi, India

Background: Osteoporosis is an emerging as an important cause of morbidity and disability in patients with thalassemia major. However, no definitive management guidelines exist.

Objectives: To determine the prevalence of low bone mineral density and to assess the Safety and Efficacy of zoledronic acid in patients of thalassemia major who have low bone mineral density.

Design/Method: This is a prospective, open label, single arm trial. Bone mineral density (BMD) at lumbar, hip and forearm region was performed using dual energy x-ray absorptiometry scan at baseline and after 1 year of therapy. Zoledronic acid was given in dose of 4 mg (later reduced to 1 mg) every 3 months for 4 doses.

Results: Forty patients (26 males) with transfusion dependent thalassemia with a median age 19.5 years (15-38 years) were enrolled. Six patients had bony pains; 9 episodes of prior fracture in 6 patients, 1 fracture during follow-up. Thirty five patients had low bone mineral density (<20 years-15/20 patients; ≥20 years-17/20 had osteoporosis, 3/20 had osteopenia).

Twenty seven patients were eligible for zoledronic acid treatment. Six patients were excluded during the study period (2 deaths, 3 drop outs, 1 hypoparathyroidism). At the start of the study, 9 patients received a first dose of 4 mg. Four patients developed grade 4 hypocalcemia (3 of these developed tetany) and 2 developed infusion related toxicity. Dose for all subsequent patients was thus revised to 1 mg.

One mg dose was well tolerated. At the end of one year, bone pains had completely resolved. There was significant increase in BMD at lumbar (p=0.002) and forearm regions (p=0.04). BMD also increased significantly at intertrochanteric area (p=0.041) and showed an improving trend at neck (p=0.052) and trochanter (p=0.093). The % change in BMD at one year was 3.7±3.2.

Conclusion: Low bone mineral density is widely prevalent in patients with transfusion dependent thalassemia. Zoledronic acid is an efficacious agent in treatment of low bone mineral density in these patients. Zoledronic acid produces significant adverse reactions at 4 mg dose but 1 mg dose is well tolerated.

Rapamycin for the treatment of complicated vascular anomalies

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Background: Complicated vascular anomalies can cause disfigurement, chronic pain, and organ dysfunction with significant morbidity and mortality. Despite the severity of potential complications, we lack uniform guidelines for the treatment and response documentation of children and young adults with these diseases.

Objectives: Pre-clinical and clinical data exist to support the essential regulatory function of the PI3K/Akt/mTOR pathway in vascular growth and organization, suggesting a therapeutic target for patients with complicated vascular anomalies.

Design/Method: Retrospective evaluation of 5 patients with complicated vascular anomalies. All lesions had a lymphatic component.

Results: Average age was 8 years with 3 females and 2 males. (Table 1) All patients had had previous medical treatment. Average time to response was 3 weeks. Responses included resolution of effusions in patients 1, 4 and 5; improvement of platelet count and fibrinogen in patient 3; and removal of drains after debulking procedure with decrease in leg circumference in patient 2. Side effects were limited to grade III mucositis (1 patient) which responded to decreased drug level, and grade II headache (1 patient). Three of five patients remain on rapamycin. No patient has had exacerbation of their disease.

Conclusion: The mTOR inhibitor rapamycin offers a possible treatment option for patients with complicated vascular anomalies particularly with a lymphatic
component. We presently have a Phase 2 trial evaluating the effectiveness and safety of this mTOR inhibitor in the treatment of children and young adults with complicated vascular anomalies. This study will use diagnostic and therapeutic response criteria and biologic marker analysis for evaluation.

Table/Charts:

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (at enrollment)</th>
<th>Diagnosis</th>
<th>Affected Locations</th>
<th>Previous Treatment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16 years</td>
<td>Microscopic Lymphatic Malformation</td>
<td>Bone marrow failure</td>
<td>Interferon/Chelates</td>
</tr>
<tr>
<td>2</td>
<td>6 years</td>
<td>Capillary Viral Lymphatic Malformation</td>
<td>Liver, Left lower extremity</td>
<td>LAMN, Interferon, Bevacizumab, Skeletal bone marrow biopsy</td>
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<tr>
<td>3</td>
<td>10 months</td>
<td>Kapostiphon Hemangioendothelioma</td>
<td>Abdomen, Back, Chest, Left leg, Pelvis, Renal, Ribs</td>
<td>Steroids, Vinblastine, Cyclophosphamide, Interferon, Acute, Totalization</td>
</tr>
<tr>
<td>4</td>
<td>14 years</td>
<td>Microscopic Lymphatic Malformation</td>
<td>Chyloous pleural effusion, Mediastinitis, Bone tumor</td>
<td>Chemo, Thera, Plakocan, Ligation of the thoracic duct, Colchicine</td>
</tr>
<tr>
<td>5</td>
<td>7 years</td>
<td>Microscopic Lymphatic Malformation</td>
<td>Planar effusion, Mediastinitis, Pericardial, Bone tumor, Cutaneous (shoulder/shoulder)</td>
<td>Interferon, Colchicine, Thrombocytopenic aterotization</td>
</tr>
</tbody>
</table>

(POSTER 118) NEW APPROACH IN TREATING PEDIATRIC HEMANGIOMAS
Geetha Puthenveetil, Jill Stites.
Children’s Hospital of Orange County, Orange, California, United States

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. However, the high predilection of these tumors for the head and neck region (~60%), can result in significant morbidity including airway compromise and visual impairment during the phase of rapid growth (3-6 months of life). Treatment of HOI includes steroids, alpha interferon and vincristine, which have a significant toxicity profile in the young infant. Leaute-Labreze et al described the inhibitory effect of propranolol on the growth of steroid-nonresponsive, rapidly proliferating hemangiomas.

Objectives: We aim to develop a safe and effective protocol for the treatment of HOI using a combination of propranolol and prednisone.

Design/Method: Infants with rapidly proliferating HOI were hospitalized to initiate therapy. Baseline studies included a complete blood count, metabolic panel, serum BNP, electrocardiogram, echocardiogram and MRI of the involved region. Telemetry was used for cardiac monitoring. Propranolol was initiated orally at 1mg/kg/day with oral prednisone at 2mg/kg/day and an H2 blocker. Blood pressures and blood sugars were monitored. The dose of propranolol was increased to 2mg/kg/day on day 2. The baseline studies were repeated prior to discharge on day 3. The patients were followed closely on an outpatient basis by hematologist and cardiologist. The dose of prednisone was rapidly tapered after the first 2 weeks of treatment; propranolol was continued for 4-6 months.

Results: 10 patients were treated using this protocol over the last 1 year. A change in color was noticed within the first 2-3 days, this was accompanied by softening of the tumor in all of our patients. All the patients have been successfully weaned off of prednisone within 4-5 weeks and have demonstrated a sustained decrease in size of the tumor. There was no evidence of hypotension or hypoglycemia in any of the patients.

Conclusion: Propranolol is a safe and efficacious therapeutic option for HOI. Our protocol decreases the exposure of this vulnerable population to the long term side effects of steroids including growth retardation and immune suppression. Larger studies are necessary to validate these findings.

(POSTER 120) LONGITUDINAL ASSESSMENT OF CLONAL CHANGES IN HEMATOPOIESIS IN FANCONI ANEMIA PATIENTS
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Background: Fanconi anemia (FA) is a complex disorder of increased sensitivity to DNA damage characterized by progressive bone marrow failure, variable congenital abnormalities and a predisposition to malignancy, particularly myelodysplasia (MDS) and acute myeloid leukemia (AML).

Objectives: The very high frequency of bone marrow failure and myeloid malignancy in this population offers an extraordinary opportunity to study prospectively the steps in the evolution of these processes.

Design/Method: We evaluated clonal changes in the hematopoiesis of patients with FA over time utilizing the single nucleotide polymorphism (SNP) microarray to assess acquired clonal abnormalities and X-linked clonality assays to identify changes in the hematopoietic stem cell compartment.

Results: We examined either peripheral blood or bone marrows samples drawn 1-2 years apart from three FA patients using SNP microarray, and further characterized breakpoints of previously identified abnormalities detected by chromosome analysis and FISH studies. Using X-linked clonality studies we also demonstrated clonal hematopoiesis in two females with FA and advanced marrow disease, with allele ratios of 0.100 and 1.99. In a third FA patient with bone marrow failure, serial samples revealed the evolution of hematopoietic clonality over a 5 month period with progression from polyclonal hematopoiesis with similar representation of the two alleles (ratio 58:42) to skewed representation of the two alleles of 76:26 in peripheral blood and 84:16 in bone marrow, indicating potential progressive depletion of stem cell reserve or emergence of a pre-malignant clonal population.
(POSTER 121)
INADEQUATE DIETARY INTAKE IN PATIENTS WITH THALASSEMIA

Ellen Fung, Yan Xu, Nancy Olivieri, Patricia Giardina, John Porter, Janet Kwiatkowski, Isaac Odame, Alexis Thompson, Ellis Neufeld, Jeanne Boudreaux, Charles Quinn, Felicia Trachtenberg, Elliott Vichinsky

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Background: Optimal nutritional status is imperative for growth and pubertal development in children as well as for immune function and bone health in adults. Patients with thalassemia (Thal) are known to have poor growth, immune function and bone mineralization. Low circulating levels of many nutrients have been reported, but few have assessed dietary intake.

Objectives: To assess dietary intake of key nutrients in a large contemporary sample of patients with Thal, identified through the Thal Clinical Research Network (TCRN), and compare their intake to the U.S. Dietary Reference Intakes (DRI).

Design/Method: Patients enrolled in the Thal Longitudinal Cohort study completed a validated, self-administered food frequency questionnaire [160 items, Block 2005]. Questionnaires were analyzed and nutrients defined as inadequate if less than 2/3 the DRI. Circulating 25-OH vitamin D and total body iron stores were assessed clinically and correlated with dietary intake. Age groups were defined as children (3-8 y), adolescent (9-18 yr), and adult (≥ 19 yr).

Results: 192 patients (19.3 ± 11.3, range 3–51 yrs, 101 F) completed the baseline assessment and are included. Of the total, 88.5% were transfused (70.7% B-thal, 11.2% E-B-thal), the remainder non-transfused (B-thal, HbH, HbH/CS, E-B-thal). The remainder non-transfused (B-thal, HbH, HbH/CS, E-B-thal).

Conclusion: Nutrient intake declined by age group (p < 0.01) particularly for vitamin A, C, E, folate, iron, calcium and magnesium. The only nutrients for which >90% of patients consumed adequate amounts were thiamin, niacin, riboflavin, vitamin B6, D and selenium. Over half the sample took additional supplements of calcium and vitamin D, though 25-OH vitamin D remained < 30 ng/mL in 61% of patients. Dietary iron intake did not correlate with total body iron stores.

(POSTER 122)
RISK OF HEART RATE OVERLOAD FOLLOWING TRANSMISSION IN PATIENTS WITH THALASSEMIA

Anurag Agrawal, Edmund Hsu, Keith Quirolo, Lynne Neumayr, Heidi Flori

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Background: Standard of practice recommends slow transfusion (i.e., 5 ml/kg over 4 hours) for children with hemoglobin levels less than 5.0 g/dL secondary to severe chronic anemia (SCA). Due to the theoretical risk of transfusion-associated circulatory overload (TACO),1

Objectives: To provide evidence as to what transfusion rate in children with SCA would be safe, our Pediatric Intensive Care Unit (PICU) has been utilizing a more liberal transfusion practice intermittently for the last ten years.

Design/Method: We reviewed all patients admitted to the PICU over the past ten years with hemoglobin levels of less than 5.0 g/dL, excluding patients with a history of TACO. Hemoglobin levels were averaged over 4 hours, and we documented vital signs including heart rate, respiratory rate, systolic and diastolic blood pressure, oxygen saturation and inputs/outputs as well as diuretic usage and chest radiograph findings to analyze for signs of TACO. We further compared these patients to an age and diagnosis matched control group that received slow transfusions.

Results: No patient had an adverse event or changes in their vitals consistent with TACO. Heart rate dropped significantly (p < 0.001) although not statistically different from the control group (p = 0.28). No patient in either group had a new oxygen requirement or worsening of their chest radiograph. Diuretics and oxygen supplementation were used in both groups empirically only.

Conclusion: Both the study patients and control group had a statistically significant drop in heart rate with transfusion, emphasizing the safety of any rate of transfusion in our population. One similar retrospective review with an equivalent safety profile has been published.2 Rate of transfusion must be based on multiple factors including convenience, timeliness of procedures and transport to an appropriate care facility, risk of alloimmunization and wastage of blood, stress for the family and need for PICU monitoring. TACO appears a theoretical risk in the normal pediatric population; therefore, slow transfusion is likely an unwarranted anecdotal practice for children with SCA.3

(POSTER 123)
IMMUNOLOGICAL FUNCTION IN CHILDREN WITH FANCONI ANEMIA

Kusiani Myers, Stella Davies, Parinda Mehta, Melinda Butsch-Kovacic, Robin Mueller, Jack Blessing

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Background: There has long been clinical suspicion that children with FA have an increased frequency of infection in excess of that due to neutropenia.

Objectives: Only a handful of small studies focusing on the immune system of FA patients have been published.

Design/Method: We have performed a detailed assessment of lymphocyte numbers and function in 10 children with FA, summarized in the table below.

Results: Abnormalities were found in nine of the ten children studied, and only one child was normal in all parameters tested. Five of the ten children had reduced numbers of B cells with abnormal phenotype, and T cell numbers were reduced in one. T cell proliferation studies showed an abnormal response to at least one antigen. Cytoxic T cell function was reduced in five of the ten children, and natural killer cell function was also abnormally low in five of the ten children.

Conclusion: Overall, these results suggest a previously unreported B-cell defect (both qualitative and quantitative) along with abnormal cytoxic function, indicating a significant B and T lymphocyte heterogeneity in some deficient FA patients. We are currently performing a prospective longitudinal evaluation to determine if this immune deficiency is fixed at birth or rather progressive secondary to bone marrow failure.

(POSTER 124)
EVALUATION OF IRON CHELATION THERAPY IN B-THALASSEMIC PATIENTS IN ZAGAZIG UNIVERSITY HOSPITAL

Sherif Badawy, Tamer Hasan Hassan, Mervat Abd Allah Hesham, Mohamed Ahmed Badr

Zagazig University Hospital, Zagazig - Egypt, Al Sharkia, Egypt

Background: Iron overload causes most of the mortality and morbidity associated with thalassemia. One of the most important practical problems with chelation therapy, is accurate assessment of body iron burden to evaluate effectiveness of iron chelators.

Combined treatment (deferoxamine&deferoxipron) have increased chelation efficacy and allow drug doses and toxicity to be reduced and number of days of deferoxamine infusion to be decreased, improving compliance and quality of life.

Objectives: Evaluation efficacy&safety of (deferoxamine&deferoxipron) in reducing transfusional iron overload compared to each drug alone and associations (survival,complications&ferritin levels).

Design/Method: Patients aged ≥8 y included in the study.Patients PRBCs /3–4 weeks to maintain Hb >9 g/dl. Patients were on desferrioxamine before study, randomized into 3 groups (50 each): Group(I) daily deferipron, desferrioxamine twice weekly, Group(II): daily deferipron, Group(III): desferrioxamine 5 days/week.

Deferoxamine (40 mg/kg/day). Deferipron (75 mg/kg/day). Patients had: history, questionnaire on chelation therapy, reasons for non-compliance, side effects, life activities, transfusion regimen. CBC monthly, serum ferritin levels, Liver and kidney functions, Blood glucose level, Serum calcium and phosphorus/3 months and T3, T4, TSH, LH, FSH, Echocardiography, Bone density Auditory and visual examination twice.

TABLE 1: COMPARISON BETWEEN PATIENTS RECEIVING COMBINED CHELATION THERAPY VS INDIVIDUAL CHELATION THERAPY
Results: Highly significant reduction in serum ferritin levels after chelation therapy in all groups. Reduction higher in group I but this difference statistically nonsignificant. Hypogonadism and growth impairment were the commonest disease complications in our study (38.7% and 36% respectively). Osteoporosis & osteopenia(34.3% and 28.7% respectively). Apart from hypogonadism, the prevalence of other endocrinal complications was much less common. Growth impairment was significantly higher in group III with no significant difference in disease complications among groups. Gastrointestinal upset was the commonest complication in group II. Local reactions were the commonest complication in group III and both complications were lower in group I. Group II and group I were more compliant to chelation therapy but difference was statistically non significant. Non-compliant patients(compliance less than 50%) showed increase in their serum ferritin levels in all studied groups. In compliant patients the reduction in serum ferritin levels was higher in group I and III than in group II but difference was statistically non significant.

Conclusion: We concluded that combined chelation therapy regimen of deferoxamine and deferiprone is more effective, less toxic and well tolerated in comparison to either drug alone.

(POSTER 125) HEREDITARY FOLATE MALABSORPTION DUE TO MUTATION AT THE ARGinine 376 RESIDUE; SUBSTRATE-SPECIFIC CHANGES IN Function and pH-DEPENDENCE

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Background: Hereditary folate malabsorption (HFM) is an autosomal recessive disorder caused by mutations in the proton-coupled folate transporter (PCFT). Infants present with failure to thrive, anemia, diarrhea, immune deficiency with severe infections, and seizures.

Objectives: To assess the functional properties of a mutant carrier (R376Q) identified in a patient with HFM.

Design/Method: Genomic DNA was extracted from peripheral blood. Polymerase chain reaction (PCR) was performed and PCFT genomic fragments containing exons 25, and flankin introns were purified on agarose gels and sequenced. An expression vector which expresses C-terminus-HA-tagged wild-type PCFT and DNA constructs containing various substitutions at the R376 residue (R376Q, R376W, R376C, R376E, R376A, R376H or R376K) were generated. HEK293A cells were transfected with wild-type PCFT, mock (vector alone), or a PCFT mutant constructs. Forty-eight hours later influx of [3H]folate substrates was assessed. PCFT protein expression and accessibility at the plasma membrane, were assessed by western blot and PCFT surface biotinylation.

Results: Loss of function for R376Q-PCFT-mediated transport was comparable among a variety of substrates (folate acid, 5-methyl-tetrahydrofolate, 5-formyl-tetrahydrofolate) and methotrexate; however, pemetrexed transport was better preserved. Influx of 5-formyltetrahydrofolate mediated by the mutant carrier was markedly decreased over a broad pH range; however, pemetrexed transport was better preserved. Influx of 5-formyltetrahydrofolate-PCFT-proton translocation rate. In compliant patients the reduction in serum ferritin levels was higher in group I and III than in group II but difference was statistically non significant.

Conclusion: We concluded that combined chelation therapy regimen of deferoxamine and deferiprone is more effective, less toxic and well tolerated in comparison to either drug alone.

(POSTER 127) NEUTROPHIL FUNCTION IN PATIENTS WITH INHERITED BONE MARROW FAILURE SYNDROMES

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Background: The Inherited Bone Marrow Failure Syndromes (IBMFS) comprise a group of rare disorders including Fanconi anemia (FA), Dyskeratosis congenita (DC), Diamond-Blackfan anemia (DBA) and Shwachman-Diamond syndrome (SDS). One common feature is single or multi-lineage bone marrow failure, which may include neutropenia. Previous studies reported decreased neutrophil chemotaxis in patients with SDS; there are no reports of respiratory burst activity in any of the IBMFS.

Objectives: To determine whether neutrophil functions are abnormal in IBMFS patients.

Design/Method: We studied 43 patients (FA = 11; DC = 28; SDS = 18; SDS = 10) and 56 healthy family members (FA = 13; DC = 28; SDS = 8; DBA = 7). Samples were collected at the NIH Clinical Center shipped on ice to Toronto, and analyzed within 24 hours. We also assayed 12 healthy control samples immediately after collection. Neutrophils were stimulated with Phorbol 12-myristate acetate and N-formyl-methyonyl-leucyl-phenylalanine, and respiratory burst analyzed by reduction of Cytochrome c or Dihydro-Rhodamine. Neutrophil chemotaxis was assayed in Transwell chambers. Student t-tests were used to compare results between patients and healthy family members; p < 0.05 was significant.

Results: No chemotactic activity was detected in neutrophils from patients or family members. Analysis of Cytochrome c reduction did not reveal any statistically significant difference in the degree of fMLP or PMA-driven respiratory burst between each of the IBMFS subgroups and their respective family members. DBA patients had a higher degree of fMLP-driven DHR reduction than their healthy family members (p = 0.04). Statistical significance was lost when all healthy family members were combined into one control group. In addition, there was no statistical difference in respiratory burst activity between any other BMFS and the pooled control group.

Conclusion: Our study failed to identify significant differences in neutrophil respiratory burst activity between IBMFS patients and healthy family members, using shipped specimens. Impaired neutrophil chemotaxis previously reported in SDS patients may be due to comparison of shipped patient samples with fresh laboratory controls. Clearly, neutrophil function must be analyzed in fresh samples. This constitutes a barrier to performing collaborative neutrophil function studies using a single standardized laboratory.

(POSTER 126) TRENDS IN PHYSICIAN RESOURCES FOR CANADIAN PEDIATRIC HEMATOLOGY ONCOLOGY PROGRAMS FROM 1999 TO 2009

Jacqueline Halton, Jack Hand, Pati Byron, Judy VanCleave, Michael Leaker, On Behalf Of The C17 Human Resources Committee

Children’s Hospital of Eastern Ontario, Ottawa, Ontario, Canada

Background: Appropriate physician resources are fundamental to the coordination and management of pediatric hematology oncology programs (PHOPs). A Human Resources Committee of the C17, the national network of Canadian academic pediatric hematopathology oncology programs, was established to develop a system to obtain accurate and comprehensive data to analyze and plan for the cancer treatment workforce. The Pediatric Oncology Group of Ontario previously established a ratio of one oncologist to 15 newly diagnosed patients with malignancy.

Objectives: To report the trends in physician workforce in Canadian academic PHOPs and establish ideal ratios for hematology and bone marrow transplant (BMT) physicians.

Design/Method: A survey was sent to the C17 Directors of the PHOPs at the 17 pediatric tertiary care centres treating children with cancer and blood disorders. Five surveys were conducted over a 10-year period. Information was obtained for oncology, hematology and BMT workload. Information was obtained on physician demographics, full time equivalent (FTE) positions, time spent in clinical, research, education and administrative activities. The Directors were asked what “ideal” workforce was needed to support their program.

Results: Since 1999, the physician workforce has increased from 71 FTE to 112 FTE positions with the distribution being 71 oncologists, 30 hematologists and 11 BMT physicians. The average oncologist to patient ratio was 1:17.9. The average ratio of physician to transplant patients was 1:19.5. The current ratio of hematologist/oncologist was 1:2.4. The 2009 job profile showed the median time spent on clinical activities by each Program was 60% clinical, 15% education, 20% research and 10% administration. The “ideal” physician FTE number to support PHOPs was 122 FTE.

Conclusion: After assessing workload, models of care, and “ideal” physician FTE required per program, the Human Resources Committee recommended the following: For academic programs a ratio of 1:15 be accepted for oncoligist to newly diagnosed patients with malignancy and a similar 1:15 ratio for BMT physicians. For every 2 oncologists 1:0.5 hematologist is the minimum ratio required. These guidelines have been accepted by the C17 and should be adopted across academic institutions as a national standard.
(POSTER 128)
THE FIRST REPORT OF A SOLID TUMOR IN SHWACHMAN-DIAMOND SYNDROME (SDS): A CLINICAL AND GENETIC ANALYSIS

Arif Manji, Santhosh Dhanraj, Christopher Anath, Sally-Lin Adams, Stephen Scherer, Runjan Chetty, Alice Wei, Yigal Dror

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Background: SDS is characterized by hypoplasia of the bone marrow and exocrine pancreas. An association with solid tumors has not been reported previously. Objectives: The aim of this study was to review the clinical data of a patient with SDS who developed pancreatic adenocarcinoma at an unusually young age and to identify novel molecular alterations which may have led to the malignant transformation. Design/Method: All 35 cases of SDS in the Canadian Inherited Marrow Failure Registry (CIMFR) were assessed for the occurrence of solid tumors; one patient was identified and reviewed. Genomic analysis of copy number alterations (CNA) and loss-of-heterozygosity (LOH) in the tumor and germline DNA was performed using the Affymetrix SNP Array 6.0, in addition to tumor immunohistochemical staining. Results: At the age of 38, this male patient was diagnosed with duodenal adenocarcinoma of the pancreas, which similar to the bone marrow is considered universally affected in SDS. His diagnosis of SDS was made at 13 years of age due to short stature, neutropenia and a hypocellular bone marrow with c.183_184TA>CT/c.258+2T>C double heterozygosity on SBDS analysis. His exocrine pancreatic dysfunction was not severe as enzyme replacement was not required. 86 CNVs and regions of LOH were identified in the tumor; within these segments, 23 genes were identified as having a possible involvement in carcinogenesis. Alterations of particular interest included the somatic amplification of two important oncoproteins (CAPN13, CDKN2C) and LOH in regions containing two tumor suppressor genes (FAF1, FGFR2). In the patient’s germline, amplification of one oncogene (PIK3CA) and deletions involving two tumor suppressor genes (CTNNA3, LGALS9C) were identified. Immunohistochemical staining of the pancreatic tumor revealed nuclear cyclin D1 expression and loss of E-cadherin expression in invasive areas. Conclusion: The relatively young age at cancer diagnosis and specific involvement of the pancreas make an association between pancreatic adenocarcinoma and SDS likely, thereby broadening the clinical phenotype of the disease. Similar to leukemia in SDS, the pancreatic cancer developed in hypoplastic tissues. This study identified several genetic alterations which may improve survival and adaptation of malignant clones among this population. Genomic analysis of copy number alterations may provide a possible mechanism for tumor expansion in SDS.

(POSTER 129)
EFFECT OF IMMUNE THERAPY ON PNH CLONE SIZE AND BONE MARROW CELLULARITY

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Background: Paroxysmal Nocturnal Hemoglobinuria (PNH) is a rare blood disorder with multiple clinical manifestations including hemolysis, thrombosis and aplastic anemia (AA). It is caused by a mutation of the phosphatidylinositol glycan A (PIG-A) gene causing the disruption of the glycosylphosphatidylinositol (GPI) synthetic pathway and subsequent deficiency of all GPI-anchored proteins on the cell surface. This change of the cell surface alters the affected cells’ survival. Immune therapy with anti-thymocyte globulin (ATG) has been shown to be more efficacious in patients with PNH associated AA through unclear mechanisms. There is no data in the current literature examining the PNH clone size before and after immune therapy in pediatric patients. Objectives: We describe the effect of immune therapy on PNH clone size and bone marrow cellularity in our patient. Design/Method: Six pediatric patients with AA with detectable PNH clones were treated with ATG, cyclosporine and steroids. Two patients had >1% clone size, satisfying the diagnosis of PNH. We present data on the patient with the largest clone and describe the response to immune therapy. PNH clone size on peripheral blood was measured pre and post therapy. Results: A 13 year old male with type 1 diabetes, presented initially with fatigue, easy bruising and pancytopenia. Initial RBC and WBC PNH clone by CD55/CD59 flow cytometry was 6.24% and 23.16% respectively. Bone marrow cellularity 1 month post therapy was 50% and the patient remains transfusion independent. Conclusion: Immune therapy with ATG, cyclosporine and steroids remains an excellent therapeutic strategy for pediatric patients with AA with a detectable PNH clone, as seen in our cohort. However, the size of the clone remains unaltered post therapy and therapies to reduce/eliminate the clone remain under investigation.

(POSTER 130)
FREQUENCY OF MINOR BLOOD GROUP ANTIGENS AMONG HISPANIC BLOOD DONORS IN SOUTH TEXAS

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Background: The frequency of minor blood group antigens has not been previously described among Hispanic blood donors. Objectives: In this study, we describe the prevalence of minor blood group antigens among Hispanic donors of Mexican-ancestry from South Texas. Design/Method: A retrospective cohort study was performed using the South Texas Blood and Tissue Center (STBTC) donor database, LifeTrak, from 1/1/1997-1/1/2009. Blood donors self-identified race and ethnicity. Caucasian and Hispanic donors were eligible. Donors who did not have minor antigen testing performed were not included. Results: For the study period, the STBTC reported 299,047 Hispanic blood donors of Mexican ancestry. Minor antigen testing was performed in 10% (74,140/779,409) of all donors; 8% (25,394/299,407) of Hispanic donors and 11% (42,891/392,074) of Caucasian donors. Hispanic donors were significantly more likely to express blood type ORh+ than Caucasian donors (13,920/25,394, 55% v 14,974/74,140, 35%; p < 0.001). Of the minor antigens, expression of RhE also differed significantly between Hispanic and Caucasian donors (4,706/11,744, 40% v 5,416/19,497, 28%; p < 0.001). The prevalence of other antigens were similar and reported in Figure 1. The RhDcE haplotype was most common among Hispanic donors and differed significantly from Caucasian donors, 50% (3,589/7,176) v 36% (3,022/8,296); p < 0.001. The RhDcE haplotype was most common among Caucasian donors (39%, 3,260/8,296 v 27%, 1,903/7,176). The RhDcE haplotype, common in African-Americans, was the least common haplotype in both groups with a similar frequency (16%, 1,354/8,296 v 15%, 1,041/7,176). Conclusion: Differences exist in the frequency of minor blood antigens among Caucasian and Hispanic blood donors in South Texas. Tables/Charts:

(POSTER 131)
NOVEL G6P3C MUTATION AND CLINICAL COURSE IN A CHILD OF DOMINICAN ANCESTRY

Lalit Bansal, Kaa Boztug, Howard Ratech, Thomas Moulton

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Background: Severe congenital neutropenia (SCN) is a genetically heterogeneous condition. Mutations in genes encoding neutrophil elastase/ELANE, HAX1, GCSF3 and WAS have been described in SCN patients. Recently, a syndromic variant associated with SCN and complex developmental defects has been described, caused by mutations in gene encoding glucose-6-phosphatase catalytic subunit 3 (G6P3C). Only a limited number of G6P3C-deficient patients have been described. Objectives: We describe the molecular and clinical phenotype in a patient with SCN bearing a novel G6P3C mutation. Design/Method: Retrospective chart review. Results: A 9 year old male of Dominican ethnicity presented with fever at 7 weeks of age and was found to have SCN. Initial clinical examination revealed dysmorphic
features with frontal bossing, upturned nose, recessed chin and triangular faces. Skin was thin with prominent superficial veins over the chest and extremities with bilateral undescended testis and right inguinal hernia. Echocardiography revealed a large ASD. A syndromic disease was suspected, but no overt chromosomal abnormalities were detected.

The patient was started G-CSF at 11 week of age at 5.8mcg/kg/day given 3 times a week (2.5mcg/kg/day). His G-CSF requirement was 2.8 to 3.2mcg/kg/day until 8 years of age and then increased to ~6mcg/kg/day to maintain adequate ANC. He developed anti-neutrophil antibodies at 3 years of age and his ANC ranged from 48–2000 during treatment. He had intermittent anemia, thrombocytopenia and splenomegaly. The anemia was treated with iron therapy with some response. Thrombocytopenia, initially for 2-3 weeks at a time, has now persisted for over a year, with the lowest value 13,000/mcL. He has had multiple episodes of otitis media and pneumonia, respectively, usually when the ANC was >500. Recent bone marrow shows acquired mild myelofibrosis. Cytogenetic evaluation for MDS and other leukemias were negative. His height and weight are at 10th and 5th percentile respectively.

Recently he was revaluated for specific gene mutation which was positive for a novel splice site mutation of the G6PC3 gene. **Conclusion:** This case is the first to report G6PC3 deficiency with a novel mutation in a child with Dominican ancestry. In addition, persistent, sometimes severe, thrombocytopenia and myelofibrosis are reported for the first time.

**Background:** Moyamoya syndrome following cranial irradiation is a documented yet rare complication in children, which is associated with stroke. Previous studies have demonstrated moyamoya syndrome as a long term complication of radiation.

**Objectives:** To report the existence of moyamoya syndrome as a short term complication of radiation and risk factor for symptomatic stroke.

**Design/Method:** Case report. A literature search of pediatric patients with radiation induced moyamoya to identify risk factors for its development. To estimate the incidence of symptomatic stroke secondary to radiation induced moyamoya.

**Results:** A six year old female presented with complex partial seizures nine months after cranial irradiation and chemotherapy for a suprasellar germ cell tumor. CT brain revealed an acute left parietal infarct. Work up for hypercoagulability was negative. MRA revealed multisegmental occlusive disease of bilateral internal carotid arteries, suggestive of moyamoya syndrome (Fig.). Literature review identified patients radiated for optic pathway gliomas at highest risk for development of moyamoya. In a study, of the 12 patients with moyamoya syndrome following cranial irradiation, 9 were found to have symptomatic strokes. Patients who received radiation directed to the Circle of Willis at an age less than five years are most likely to develop moyamoya syndrome.

**Conclusion:** Radiation induced moyamoya may rarely occur early (< 12 months following radiation therapy). The highest incidence of radiation induced moyamoya appears to be in patients with optic glioma, suggesting that screening with MR angiography may be useful. Radiation induced moyamoya syndrome is a rare yet important cause of stroke in children.

**Background:** Pulmonary Hypertension (PH) is a serious complication associated with adverse outcome in adults with sickle cell disease (SCD). Endothelin (ET), a potent vasoconstrictor, is elevated in SCD and may play a role in pathological processes including PH. ET-receptor antagonists such as bosentan may be beneficial in such patients. While bosentan has been used therapeutically in adult SCD, pediatric studies including PH.ET-receptor antagonists such as bosentan may be beneficial in such patients. While bosentan has been used therapeutically in adult SCD, pediatric idiopathic PH and PH associated with congenital heart disease, limited data exists for its use in pediatric patients with SCD and PH.

**Objectives:** To report the use of bosentan in a 12 year old male-LW, with homozygous- SCD, PH and cor-pulmonale.

**Design/Method:** Clinical course: Chronic PRBC transfusion due to middle cerebral artery stenosis(MCA), progressive exertional dyspnea and exercise intolerance. Sildenafil was initiated at 25mg PO twice daily then increased to four times daily after PH diagnosed on cardiac catheterization. Eight months on sildenafil, LW developed acute cardiopulmonary deterioration, worsening PH and right heart failure. World Health Organization(WHO) functional class IV. Bosentan was initiated at 62.5mg dose oral twice daily then increased to 125mg bid.

**Results:** Mean pulmonary artery(PA) pressure(PAP) 47mmHg. PA wedge pressure 15mmHg. Pulmonary vascular resistance 8.86 Wood Units*mmH2O. Initiation of sildenafil: Tricuspid regurgitant jet(TRJ) and right ventricular pressure (RVP), 4.8ms and 102mmHg respectively. Initiation of bosentan: TRJ and RVP 4.75ms and 100.3mmHg. Bosentan month 5. TRJ and RVP 3.67ms (23% reduction) and 63mmHg (37% reduction). WHO functional class II. No adverse events associated with bosentan.

**Conclusion:** Bosentan may be an effective agent in pediatric patients with SCD and PH. Clinical trials to evaluate bosentan in pediatric SCD patients with PH or other hemolyis-associated mechanisms are indicated.
Background: Evidence-based data regarding treatment of venous thromboembolism (VTE) in pediatric patients is scant, extrapolated from adult studies, and comprised of consensus guidelines. Despite the rising incidence of VTE in pediatric patients, no data exists for VTE risk assessment and prophylaxis in hospitalized children. In December 2008, Cleveland Clinic Children’s Hospital instituted the Pediatric Anticoagulation Management Program (PACMP), an initiative aimed at standardizing prophylaxis, diagnosis and treatment of thromboembolic events. The PACMP applies to admitted patients age ≥16 or <16 with weight >70 kg or BMI ≥30. Patients satisfying these criteria should be risk-assessed and placed on VTE prophylaxis as recommended.

Objectives: This observational study was designed to examine behaviors surrounding VTE risk assessment and prophylaxis in a tertiary children’s hospital.

Design/Method: Distribution of risk assessment categories was extracted from the electronic medical records (EMRs) of patients age < 18 years admitted to the Children’s Hospital during the same 3-month period preceding and immediately following PACMP implementation.

Results: Of 1349 admissions in 2009, 489 were risk-assessed at admission, for a compliance rate of 36%. Of those assessed, 24 patients were assessed as “low risk,” 13 as “moderate risk,” 3 as “high risk,” and 512 as “age < 18” (it is possible for a patient to be assessed more than once per admission). Of patients assessed as “age < 18,” 47 were age ≥16 and 12 were < 16 with weight >70 kg or BMI ≥30. According to the guidelines, these patients are potentially at increased risk of VTE. All patients who fell into this increased risk category were assessed as follows: 59 as “age ≤18,” 10 as “low risk,” 6 as “moderate risk,” 3 as “high risk”; 144 (64%) were not assessed at all.

Conclusion: Implementation of guidelines for VTE prophylaxis has resulted in greater awareness of this topic at our institution. Expectedly, compliance is not optimal; a resident survey indicates that this part of the admission orders is just forgotten, and most residents do not feel qualified to assess VTE risk. This study has identified opportunities for improvement in our institutional practices, particularly with regard to order entry in the EMR and educational initiatives.

(PAPER 135) VTE PROPHYLAXIS IN A CHILDREN’S HOSPITAL: PRACTICES AND OPPORTUNITIES FOR IMPROVEMENT

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Background: Evidence-based data regarding treatment of venous thromboembolism (VTE) in pediatric patients is scant, extrapolated from adult studies, and comprised of consensus guidelines. Despite the rising incidence of VTE in pediatric patients, no data exists for VTE risk assessment and prophylaxis in hospitalized children. In December 2008, Cleveland Clinic Children’s Hospital instituted the Pediatric Anticoagulation Management Program (PACMP), an initiative aimed at standardizing prophylaxis, diagnosis and treatment of thromboembolic events. The PACMP applies to admitted patients age ≥16 or <16 with weight >70 kg or BMI ≥30. Patients satisfying these criteria should be risk-assessed and placed on VTE prophylaxis as recommended.

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Conclusion: Implementation of guidelines for VTE prophylaxis has resulted in greater awareness of this topic at our institution. Expectedly, compliance is not optimal; a resident survey indicates that this part of the admission orders is just forgotten, and most residents do not feel qualified to assess VTE risk. This study has identified opportunities for improvement in our institutional practices, particularly with regard to order entry in the EMR and educational initiatives.
Objectives: A workshop at the 2008 ASPHO Annual Meeting functioned as the first step in a systematic needs assessment of the particular challenges to satisfaction and success in the middle and senior phases of career development for pediatric hematologist/oncologists.

Design/Method: 61 ASPHO members attended and were randomly distributed to small discussion groups based on self-identified career stage. Groups completed “Challenge Forms” for each issue identified as pertinent to their own stage of professional development.

Results: A total of 71 forms with usable data were generated. The largest number of challenges described (26) clustered around themes of “Work-Life Balance” followed by “Transition and Succession” (18), “Financial & Practices” (15) and “Keeping Up to Date” (13). Mid-career groups were more likely to identify Work-Life Balance challenges while senior-stage groups were more likely to articulate Succession and Practice Management challenges.

Conclusion: There was remarkable coherence in the challenges reported by both Mid-career and Senior pediatric hematologist/oncologists. It is noteworthy that the type of challenge most frequently cited by both Mid-career and Senior groups is that of work-life balance. Mid-career groups were more likely to identify Work-Life Balance challenges while senior-stage groups were more likely to articulate Succession and Practice Management challenges.

Objectives: To discuss the clinical triad of osteomyelitis, DVT, and PE.

Methods: An 13 y/o boy was admitted with back pain and fever four days after sustaining minor trauma to his lower back. Upon presentation he was found to be febrile to 38.2°C and in moderate amount of distress secondary to pain. Musculoskeletal exam revealed significant tenderness to palpation of the paraspinal muscles and spinous processes throughout his lumbar spine. Complete neurologic exam was unremarkable. Laboratory studies included a WBC count of 21 x 10^3 (82% neutrophils and 7% bands). Blood cultures grew Staph aureus therefore vancomycin and cefazolin were begun. An MRI revealed vertebral osteomyelitis at L3. Twenty-four hours later the boy developed acute onset subinternal chest pain and hypoxia. A spiral CT scan identified bilateral segmental and subsegmental pulmonary emboli. He was immediately begun on anticoagulation with enoxaparin. Further investigation with a contrasted abdominal and pelvic CT scan revealed a thrombus located in the infrarenal inferior vena cava at the level of his previously diagnosed vertebral loci to the large veins.

Musculoskeletal exam revealed significant tenderness to palpation of the paraspinal muscles and spinous processes throughout his lumbar spine. Complete neurologic exam was unremarkable. Laboratory studies included a WBC count of 21 x 10^3 (82% neutrophils and 7% bands). Blood cultures grew Staph aureus therefore vancomycin and cefazolin were begun. An MRI revealed vertebral osteomyelitis at L3. Twenty-four hours later the boy developed acute onset subinternal chest pain and hypoxia. A spiral CT scan identified bilateral segmental and subsegmental pulmonary emboli. He was immediately begun on anticoagulation with enoxaparin. Further investigation with a contrasted abdominal and pelvic CT scan revealed a thrombus located in the infrarenal inferior vena cava at the level of his previously diagnosed vertebral osteomyelitis as well as bilateral paos abscesses.

Results: In the triad of acute osteomyelitis, deep venous thrombosis, and septic pulmonary emboli, it has been postulated that inflammation associated with osteomyelitis leads to localized endothelial damage and release of inflammatory cytokines. This process subsequently results in activation of the coagulation cascade thus predisposing patients to development of venous thrombus in the area of infection. Staphylococcal species are known to release exotoxins including coagulase, which causes clot formation by way of interaction with fibrinogen, and alpha-toxins, which cause platelet aggregation and smooth muscle spasm. Individuals with acute osteomyelitis occurring in the vertebrae, proximal extremities and pelvis are at the greatest risk of thrombus formation related to infection due the proximity of infectious loci to the large veins.

Conclusion: Clinical suspicion for deep venous thrombosis and septic pulmonary emboli should remain high in individuals with osteomyelitis occurring in proximity to the large veins.

Objectives: To understand the pathophysiology involved in thrombosis with osteomyelitis.

Results: In the triad of acute osteomyelitis, deep venous thrombosis, and septic pulmonary emboli, it has been postulated that inflammation associated with osteomyelitis leads to localized endothelial damage and release of inflammatory cytokines. This process subsequently results in activation of the coagulation cascade thus predisposing patients to development of venous thrombus in the area of infection. Staphylococcal species are known to release exotoxins including coagulase, which causes clot formation by way of interaction with fibrinogen, and alpha-toxins, which cause platelet aggregation and smooth muscle spasm. Individuals with acute osteomyelitis occurring in the vertebrae, proximal extremities and pelvis are at the greatest risk of thrombus formation related to infection due the proximity of infectious loci to the large veins.

Conclusion: Clinical suspicion for deep venous thrombosis and septic pulmonary emboli should remain high in individuals with osteomyelitis occurring in proximity to the large veins.

Background: Recent functional studies have revealed pathological platelet activation associated with intravascular hemolysis to be an important contributor to the pathogenesis of sickle cell disease (SCD) vasculopathy. In addition, our previous platelet transcriptome studies in sickle cell disease have demonstrated dysregulated arginine and polyamine metabolism leading to decreased NO bioavailability and hence contributing to endothelial dysfunction. These data suggest that platelets are regulated directly by intravascular NO levels, and indirectly by transcriptional control of intracellular arginase levels.

Objectives: We have undertaken the present pilot study to demonstrate and validate the presence of microRNAs (miRNAs) in platelets involved in proinflammatory regulation of the NO signaling pathway and platelet function.

Results: Data mining identified 280 miRNAs expressed in platelets. Out of these 49 miRNAs (27 up- and 22 down-regulated) distinguished SCD samples from normal controls, at a fold-change > 2, and 5% false-discovery rate. Prediction of target genes was performed by bioinformatics-based approaches. Some relevant miRNAs with their predicted target genes identified were: mir-630 and mir-150 (both upregulated) with targets that included arginine-glutamic acid peptide, thrombospondin type-1, and platelet glycoprotein Gp-IIIa; miR-744 (down) and miR-144 (up) with targets that included arginine-glutamic acid peptide and the NMDA receptor. Further validation of negative expression of miR-744 and miR-630 was conducted by real-time PCR. We have collected 30 more samples and will be analyzing them in the context of functional studies. The results will be updated at the meeting.

Conclusion: The present study confirms the existence of regulatory platelet miRNAs and their differential expression in SCD. Future evaluation of miRNA-target mRNA interactions will provide an experimental framework for the study of disease-specific platelet biology and potentially identify biomarkers for predicting patients at high risk of developing vasculopathy.

Background: The etiology of anti-Factor VIII (F8) inhibitor formation in Hemophilia A patients is not well understood. T cell help for B cell antibody formation through both costimulatory signaling and cytokine production is likely involved, as T effector cells (Teff) secreting IFN-γ (Th1), IL-4 (Th2), and IL-17 (Th17) are observed in mice and humans who develop inhibitors. Conversely, TGF-β (Th3) secreting regulatory T cells (Treg) may thwart inhibitor formation. Hence, inhibitor formation may be prevented by mechanisms of immune stimulation that favor the development of Treg over Teff. Treatment with the novel tolerogen E.coli Colony Forming Antigen I (CFA/I) has been demonstrated to prevent development of inflammatory diseases such as collagen induced arthritis and experimental autoimmune encephalitis via stimulation of Treg in the mouse model.

Objectives: We hypothesize that treatment with CFA/I will favor induction of Treg and prevent inhibitor formation in a mouse model of Hemophilia A.

Design/Method: F8-deficient mice were immunized with 4 doses of 2 microgram recombinant human F8 (Kogenate) at weekly intervals. 100 microgram of CFA/I was administered intranasally on days 12-14 post first F8 immunization, and plasma samples and will be analyzing them in the context of functional studies. The results will be updated at the meeting.

Results: Bethesda assays of serum obtained day 28 show significantly decreased inhibitor titer in CFA/I vs PBS treated mice (see graph). Cytokine multiplex data also showed decreased IFN-γ and IL-17.

Conclusion: Preliminary evidence suggests CFA/I therapy may prevent inhibitor development. This finding may be explained by decreased inflammatory cytokine production. Further studies such as optimum route and dosing schedule are necessary and currently ongoing.

Tables/Charts:
(POSTER 142)

THE SPECTRUM OF KAPOSIIFORM HEMANGIOENDOTHELIOMA: A REVIEW OF 107 CASES ELUCIDATES ATYPICAL PRESENTATIONS AND RISK FACTORS FOR KASABACH-MERRITT PHENOMENON

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Background: Kaposiform Hemangioendothelioma (KHE) is an uncommon vascular tumor presenting in early childhood with distinctive cutaneous features and often Kasabach-Merritt phenomenon (KMP), a severe thrombocytopenia resulting from intralesional platelet activation and trapping and is associated with fatal complications.

Objectives: The purpose of this study was to review our large series of patients to better understand the spectrum of this tumor, including atypical presentations and prediction of KMP.

Design/Method: We retrospectively reviewed the clinical, imaging, histologic and laboratory data for 107 patients with KHE evaluated at a single referral center between 1991 and 2009. The diagnosis of KHE was reached by consensus based on combinations of photographs, clinical examination, imaging and histopathology.

Results: KHE occurred in a male-female ratio of 1.3:1. Initial presentation occurred in infancy in 93%: neonatal (59%), 1-3 months (16%), 3-6 months (10%), 6-12 months (8%), 1-4 years (5%) and >4 years (2%). KHE involved the torso (46%), hip/lower extremity (38%), shoulder/upper extremity (36%), and cervicofacial (33%), retroperitoneal (12%) and intrathoracic (7%) regions. 11% lacked cutaneous manifestations of KHE. Presenting signs and symptoms were: change in lesion appearance (73%), thrombocytopenia (55%), pain/decreased function (23%) and other findings (18%), including respiratory distress, heart failure, hypotonia, ascites, abdominal pain or hematocrit. Overall, 72% developed KMP (89% at presentation, 11% delayed). KHE isolated to subcutaneous tissue manifested KMP in only 31% compared to 76% of tumors invading underlying muscle, retroperitoneum or mediastium. No KMP developed in the three lesions confined to bone. By KHE location, KMP frequency was: intrathoracic 100%, retroperitoneal 85%, cervicofacial 84%, torso 79%, shoulder/upper extremity 74% and hip/lower extremity lesions 66%.

Novel presentations of KHE in patients greater than 1 year-old were not associated with KMP.

Conclusion: The largest cohort of KHE patients reviewed to date includes 28% without KMP. This lesion most commonly presents as an enlarging cutaneous lesion; however, we identified 11% without cutaneous manifestations. While most KMP is present initially, delayed presentations occur. Lesions first identified outside of infancy have increased risk for KMP upon presentation. The depth of infiltration of KHE, more than the anatomic location, predicts risk of KMP.

(POSTER 143)

FONDAPARINUX IN CHILDREN: A PROSPECTIVE, DOSE-FINDING, PHARMACOKINETIC AND SAFETY STUDY

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Background: Fondaparinux has several attractive properties including a longer half-life, lack of animal sources.

Objectives: To determine dosing and safety of fondaparinux in patients with DVT.

Design/Method: Children 1-18 years with DVT or HIT were eligible, and received fondaparinux 0.1mg/kg up to 7.5 mg once-daily. Fondaparinux levels determined via a fondaparinux-standard anti-factor Xa assay (results expressed in mg/L) were measured before and 2, 4, 12, and 24 hours after the first dose. Subsequently, levels were drawn at 4 hours twice-weekly and following dose changes. The target peak (4 hours) fondaparinux level was 0.5-1.8 mg/L. Levels outside this resulted in dose adjustments. A level at 12 hours <0.2mg/L led to twice-daily dosing. Major and minor bleeding and adverse events were recorded per pre-defined criteria. A population pharmacokinetic model was developed and simulations were performed comparing observed and predicted fondaparinux concentrations in children with those observed in adults.

Results: 24 patients from 3 age cohorts (1-5, 6-12, and 13-18 years) with 10, 7, and 7 per cohort were enrolled. The mean duration of study drug was 5.8 days (range 2–19). 87.5% of patients achieved a therapeutic level following the first dose and no patients required twice daily dosing. Three patients (12.5%) required a dose adjustment (increased in 2 and decreased in 1). Pharmacokinetic modeling demonstrated Cmax and Cmin concentrations at 0.1mg/kg once-daily dosing similar to those in adults. Two bleeding events occurred: 1) an intracranial hemorrhage which likely occurred prior to study drug initiation; 2) an episode of occult blood in the stool which led to temporary discontinuation of study drug. No other adverse events occurred.

Conclusion: Fondaparinux given once daily at 0.1mg/kg in children with DVT or HIT reliably led to the targeted pharmacodynamic effect with minimal adverse events. These results support such initial dosing in carefully selected pediatric patients for whom fondaparinux may provide a net benefit over other anticoagulants.

(POSTER 144)

COST-UTILITY ANALYSIS OF VON WILLEBRAND DISEASE SCREENING IN ADOLESCENTS WITH MENORRAGHIA

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Background: Whether to evaluate all adolescent females presenting with menorrhagia for von Willebrand disease (VWD) is unknown. In the general population, the prevalence of VWD, the most common bleeding disorder in humans, is 1%; however in adolescents with menorrhagia, it is significantly higher at 13%. In 2001 the American College of Obstetricians and Gynecologists (ACOG) recommended that all adolescent women with menorrhagia be screened for VWD reining slightly this statement in 2009.

Objectives: The primary aim of this study was to construct a decision analysis model to evaluate the cost utility of VWD testing in adolescents with menorrhagia.

Design/Method: A 20-year Markov decision analytic model was constructed to evaluate the cost utility of two strategies: testing or not testing for VWD. The model includes probabilities of remaining well, suffering an acute menorrhagia bleeding event, surgical complications, OCP complications or dying. Probabilities, costs and utilities were estimated from published literature. The hypothetical patient is a 15-year old female presenting with menorrhagia and a decision regarding whether VWD testing is made. All patients were assumed to have type I VWD, which was DDAVP responsive, and treated with oral contraceptive pills to control their menorrhagia. The Markov model cycle length was one year with the patient cycling through the model until age 35 years, an age where the menorrhagia workup includes additional evaluation for oncologic and anatomic anomalies.

Results: The cost of testing adolescents with menorrhagia for VWD was $1,790 vs. $1,251 for not testing for VWD. The effectiveness of testing in quality-adjusted life-year gained (QALY) (14.237 QALY) was similar to the VWD testing strategy (14.246 QALY). Compared to not testing for VWD, screening for VWD had an incremental cost-effectiveness ratio (ICER) per QALY of $62,791/QALY, a value typically considered economically reasonable. Using Monte Carlo analysis, testing for VWD is a cost effective strategy ~56% of the time using an accepted cost-effectiveness threshold of $100,000/QALY.

Conclusion: In adolescents with menorrhagia, it is cost effective to perform testing for von Willebrand disease prior to the initiation of oral contraceptive pills.

(POSTER 145)

CURRENT IN-HOSPITAL MORTALITY, AGE-VARYING CO-MORBIDITIES, AND HEALTH-CARE UTILIZATION FOR HEMOPHILIA IN PEDIATRIC AND ADULT PATIENTS IN UNITED STATES: FIRST REPORTED NATIONWIDE ESTIMATES

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Background: Hemophilia is a common inherited severe bleeding disorder with ~400 births/year in the US but nationwide data on in-hospital mortality, co-morbidities and healthcare utilization are not available.

Objectives: To estimate the prevalence of co-morbidities, in-hospital mortality, co-morbidities and healthcare utilization in Hemophilia A/B patients in pediatric and adult age groups.

Design/Method: National Inpatient Sample, a stratified probability sample of 20% of all US hospital discharges which has data from 1044 hospitals located in 40 states with records of 8 million hospital stays was used. Hemophilia-A and B were identified using ICD-9 code 286.0 and 286.1 respectively and sampling weights were applied.

Results: During the year 2007, 2,229 admissions with Hemophilia-A (2103) or B (451) were reported. The Table outlines age-related co-morbidities in decreasing order of frequency among Hemophilia A/B patients. No pediatric patients had a diagnosis of Hepatitis-C or HIV. Most common co-morbidity in children was central-line infection. A total of 212 in-hospital deaths were reported with only 15 deaths in children. All childhood deaths were in the neonatal period (Day 0 of life only). Intra-ventricular
hemorrhage and Newborn hemorrhage-NOS were each cited as secondary diagnoses in 33% of these patients. No pediatric deaths were reported outside the neonatal period.

Conclusion: In-Hosial care of Hemophilia represents a major healthcare cost. In 2007, there were no reported pediatric hospital admissions with HepatitisC/HIV which were the most common causes of death during 1980-90s. Central line infections(pediatrics) and essential hypertension(adults) are now the most common co-morbidities in Hemophilia admissions. These changes in co-morbidities with low death rate are likely due to the comprehensive management strategies for hemophilia including the use of factor prophylaxis.

Tables/Charts:

(POSTER 146)
EXTENSIVE SUPERIOR VENA CAVA THROMBOSIS IN INFANTS AND CHILDREN: RESULTS OF CATHETER INTERVENTION
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Background: Superior vena cava (SVC) syndrome can be caused by either simple localized SVC obstruction, or extensive thrombosis of the SVC and its tributaries. Extensive SVC thrombosis (ESVCT) is extremely difficult to treat medically, and may be life threatening. The role of catheter-based therapies for ESVCT has not been well established.

Objectives: We report our institutional experience with an aggressive approach of early catheter-based treatment of ESVCT, including mechanical thrombectomy, balloon angioplasty, and stent implantation.

Design/Method: Retrospective review of ESVCT treated in our cardiac catheterization laboratory.

Results: From 01/2004 to 01/2010, 17 patients (pts) underwent catheterization (cath) at a mean age of 4.4 months (25d–11 yrs) & a weight of 5.3 kg (1.16-36) for treatment. Catheter interventions are effective at treating ESVCT in neonates & older children, but may require multiple treatment modalities over several sessions. Interventional catheterization should be considered in the management of SVC thrombosis when feasible.

(POSTER 147)
A COST-UTILITY ANALYSIS OF CRANIAL IMAGING IN NEONATES WITH HEMOPHILIA
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Background: Intracranial hemorrhage (ICH) is a concern in neonates with hemophilia. The incidence has been approximated at 3.5%; however, depending on mode of delivery, it may be considerably higher. With different sensitivities and costs of imaging modalities, there remains controversy about screening. Cost-utility analyses are ideal to address such problems.

Objectives: The primary aim of this study was to evaluate the costs and utilities of three strategies to screen for ICH in neonates with hemophilia.

Design/Method: A decision analysis model compared: 1) screening all newborns; 2) screening only after complicated deliveries (i.e., forceps or vacuum-assistance); and 3) not screening. We performed a literature search to estimate the prevalences of ICH, the probabilities of death or neurologic sequelae, and the sensitivities of imaging modalities. From published sources, we obtained quality of life (utility) and cost data on diagnostic imaging, hospital care, recombinant factor administration, neurosurgical intervention, and lost parental wages. The base-case analysis assumed a 3.5 kg term neonate would be screened with cranial ultrasound. Parameters (including imaging) were varied in sensitivity analyses. Using a societal perspective and duration of 30 days, we compared the strategies based on total costs and quality-adjusted life-days (QALDs).

Results: Costs for the three strategies were $1460 for screening complicated deliveries, $1473 for not screening, and $1565 for screening all newborns. Total utilities were found to be 0.74755 QALDs for screening complicated deliveries, 0.74585 for not screening, and 0.74893 for screening all. The strategy of not screening newborns was dominated by screening complicated deliveries (higher cost and lower utility). Conversely, the incremental cost-effectiveness ratio between screening all newborns and screening complicated deliveries was $75,802/QALD. This pattern held with CT scan or MRI as well.

Conclusion: In newborns with hemophilia, the strategy of screening newborns after complicated deliveries appears to be the most cost-effective. This remained as long as screening led to shorter durations of factor replacement and hospital stays. While this offers evidence to guide the care of newborns with hemophilia, it also highlights that the high cost of factor replacement and hospital admission make the economic evaluation of hemophilia programs very challenging.

(POSTER 148)
SEVERITY AND PATTERN OF EPISTAXIS IN CHILDREN WITH VON WILLEBRAND DISEASE (VWD) OR A PLATELET FUNCTION DISORDER (PFD)
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Background: Epistaxis occurs frequently in healthy children as well as in children with an inherited mucocutaneous bleeding disorder. There is no standardized approach to determine which children with epistaxis should be screened for an underlying bleeding disorder. Determining the features of epistaxis which are suggestive of an underlying hemostatic defect would enable timely referral of patients and appropriate laboratory testing.

Objectives: To describe the severity and pattern of epistaxis in children with VWD or a PFD using standardized scores.

Design/Method: 107 pediatric bleeding questionnaires (PBQs) administered to children with a known diagnosis of VWD (79%) or a PFD (21%) at The Hospital for Sick Children were retrospectively reviewed. Patients were scored from 0-4, depending on clinical severity of their most severe epistaxis episode (0: no epistaxis or trivial, ≤5yr; 1: >5yr or >10 min duration; 2: consultation with a healthcare professional; 3: packing, cautery or antimicrobials; 4: blood transfusion, replacement therapy or desmopressin). Patients were also questioned regarding family

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History and seasonal correlation of epistaxis. Patients who had ≥5 episodes of epistaxis/yr were eligible to be graded as mild or severe using the epistaxis scoring system of Katsanis7. Results: Epistaxis was present in 66 patients (62%), of which 12 (18%) scored 0 for trivial epistaxis, 16 (24%) scored 1, 10 (15%) scored 2, 10 (15%) scored 3 and 18 (27%) scored 4. There were 39 males (59%) and 27 females (41%), median age 12 yrs (range: 6 mo–18 yrs). 49 (74%) had WVD and 17 (26%) had a PFD. A positive family history of epistaxis was documented in 44 patients (67%) and 52 patients (79%) had no seasonal correlation of epistaxis. Epistaxis lasted >10 min in 41 patients (62%). 33 patients (50%) were graded as having severe epistaxis and 30 of these (91%) had clinically significant epistaxis (PBQ score ≥2).

Conclusion: This study highlights features of epistaxis in children with an underlying bleeding disorder: need for medical/surgical intervention, positive family history of epistaxis, lack of seasonal correlation and epistaxis >10 min.

References:

(background)

Diagnostic Outcome of Preoperative Coagulation Testing on 791 Children
Neha Bhasin, Robert Parker
Stony Brook University Medical Center, Stony Brook, New York, United States

Background: The value of routine preoperative coagulation testing in asymptomatic children prior to tonsillectomy and adenoidectomy has been questioned. However, there is variability in this practice even though these tests infrequently reveal significant abnormalities predictive of an increased risk of bleeding.

Objectives: To determine whether routine preoperative coagulation testing was effective in identifying those children likely to have a clinically significant coagulation abnormality. An additional objective is to document the causation of abnormal PT and/or aPTT in this population.

Design/Method: Clinical and laboratory data, including, personal and family history of bleeding, results of initial coagulation studies and the results of the consultative laboratory evaluation was extracted by chart review for 855 consecutive patients referred to the pediatric hematology/oncology service at Stony Brook University Medical Center for the years 1994-2009 for the evaluation of an elevated prothrombin time (PT) and/or activated partial thromboplastin time (aPTT) noted on pre-operative testing.

Results: Of the 855 charts reviewed, 791 (92.5%) contained sufficient data for analysis. Of these patients, 397 (50.1%) had a laboratory abnormality identified on further testing; 281 (281/791 = 35.5%) had normal PT and aPTT on consultative laboratory testing. A clinically significant coagulation abnormality was identified in 27 patients; mild-to-moderate von Willebrand disease (n = 21), followed by low F.VII (n = 3), hemophilia A (n = 2) and liver disease (n = 1). This group represented 3.4% of the total population and 6.8% of those patients with an identified abnormality. For the 370 patients with clinically insignificant abnormalities causing a prolonged PT and/or aPTT, the most common diagnosis was a lupus anticoagulant (n = 117, 29.5%) or a “presumed” lupus anticoagulant (n = 145, 36.5%). A positive bleeding history was documented in 256 patients (256/791 = 32.4%). Of these, only 107 (41.2%) had an abnormality identified on further work-up. Only 16 patients with a positive bleeding history (16/27 = 59.3%) had a clinically significant abnormality identified.

Conclusion: Routine preoperative coagulation testing identifies only a small number of children with clinical severity of PTS varies in children with VTE over a period of time. A multi-center longitudinal follow up study is urgently required to better delineate the risk-factors and natural history of PTS in children.

Background: PTS is increasingly being recognized in children with VTE. Limited information is available about the risk factors and the natural history of PTS in children.

Objectives: To study the prevalence, risk-factors and evolution of PTS in children with VTE.

Design/Method: This cross-sectional study included longitudinal data collection on all children with confirmed diagnosis of non-CNS VTE over a 6-year period with a minimal follow up of 10 months. Children with isolated pulmonary embolism were excluded. Canadian PTS classification was used to assess the severity of PTS.

Results: A total of 71 VTE events were reported in 67 children (Table 1). The prevalence of PTS was 46.4% (95% CI 34.6 to 58%). Occurrence of VTE in an outpatient setting and delay in treatment intervention (>2 days) were associated with occurrence of PTS. The longitudinal follow up of PTS patients showed that the severity of PTS varied over time in 8/32 (25%) children: 2 progressed from mild to severe while the clinical severity was reduced from severe to mild in 1 child. The remaining 5 children continued to have mild to moderate PTS with fluctuation in clinical severity scores.

Conclusion: This study for the first time showed that clinical severity of PTS varies in children with VTE over a period of time. A multi-center longitudinal follow up study in children with VTE is urgently required to better delineate the risk-factors and natural history of PTS in order to develop interventions to prevent the progression of PTS.

Background: Platelets are not only critical in establishing hemostasis but are also important cells during inflammation and immune responses. As part of the innate immunity, the complement system interacts with platelets and coagulation components. Platelet activation can induce complement activation and vice versa. Complement factor H (CFH) is a key inhibitor of the alternative complement pathway that also plays a role in platelet stimulation as shown by the platelet aggregation effect of plasma from an atypical HUS patient with CFH autoantibodies (Licht et al, Blood 2009). We have demonstrated the in vivo uptake and release of exogenous CFH in a CFH-null patient by platelets as well as the in vitro uptake of labeled CFH. It is known that platelet glycoprotein IIb/IIIa (GPIIb/IIIa) is involved in the uptake of exogenous plasma proteins such as fibrinogen via endocytosis. However, the mechanism of platelet CFH uptake and release is poorly understood.

Design/Method: To determine whether GPIIb/IIIa is involved in the uptake of CFH we incubated platelets from patients with Glanzmann thrombasthenia that lack functional GPIIb/IIIa with fluorescently labeled fibrinogen or CFH and visualized protein uptake via laser fluorescence spinning disk confocal microscopy. To investigate the release of CFH from normal platelets we stimulated quiescent washed platelets with various agonists and detected the release of specific proteins from various platelet compartments via immunoblotting.

Results: Fibrinogen uptake was absent in platelets lacking functional GPIIb/IIIa, however uptake and distribution of fluorescently labeled CFH was not affected, suggesting that the mechanism of internalization of CFH in platelets does not involve GPIIb/IIIa. Platelet stimulation studies demonstrated that platelets are able to differentially release CFH in the absence of -granule secretion and the release of other cytoplasmic marker proteins.

Conclusion: We conclude that the uptake of CFH in platelets is not dependent on GPIIb/IIIa but mediated by a distinct uptake mechanism. Furthermore, our studies show that CFH release from platelets is independent from -granule secretion and overt cytoplasmic release mechanisms. Further studies are required to elucidate the precise mechanism of uptake and release of CFH from human platelets.

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(POSTER 152)
SINGLE CENTER EXPERIENCE WITH IMPLANTED VENOUS ACCESS DEVICES IN CHILDREN WITH INHERITED BLEEDING DISORDERS

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Background: Venous access device (VAD) use in hemophilia allows improved factor therapy and fewer bleeds. However, complications including infection or malfunction limit VAD acceptance.

Objectives: To review the experience of VAD use in pediatric hemophilia patients at a single hemophilia treatment center.

Design/Method: Retrospective review of electronic medical records.

Results: Thirty-six children were identified (24 hemophilia A (67%), 11 hemophilia B (30%), and 1 factor VII deficiency (3%). Indications for VAD were primary prophylaxis in 26 patients (72%), secondary prophylaxis in 9 (25%), and on-demand therapy in 1 (3%). Cumulative VAD days was 640,575 (175.5 years). Average age of first VAD placement was 4.1 years (range 0.1–11.8 years) and average life span for 1 VAD 3.1 years (range 2 weeks–7.1 years). Nineteen patients (53%) have their original VAD with average lifespan 3.8 years (range 0.4–7.1 years); 12 patients have a 2nd VAD; 1 has a 3rd VAD; 2 have a 4th VAD; and 2 have a 5th VAD. Reasons for VAD loss are infection: 17% (11 removed) or malfunction: 27% (17 removed). The infection rate was 0.58 infections/1000 VAD days with no difference between inhibitor and non-inhibitor patients. Four patients (11% of total) accounted for 50% of VADs placed (n = 19), 51% of documented infections (n = 19, average 2 infections/1000 VAD days), and 47% removed due to malfunction (n = 8). Inhibitor patients were more likely to suffer VAD loss due to malfunction, but did not demonstrate an increased infection rate compared to non-inhibitor patients. Efficacy was excellent with 35 of the 36 patients (97%) still using the VAD for the intended purpose. No deaths occurred. No episodes of intravascular hemorrhage or life threatening bleeds were associated with VAD placement or revision.

Conclusion: VADs are safe and effective in children with hemophilia. A single VAD can function for several years with a low infection rate. A subset of our patients account for a large percentage of VAD infection and malfunction. Identification of risk factors in the subset of patients will further improve the safety profile of VAD use in hemophilia.

(POSTER 153)
EFFECT OF ROMIPLOSTIM ON PARENTAL BURDEN AND HEALTH-RELATED QUALITY OF LIFE (HRQOL) IN CHILDREN WITH CHRONIC IMMUNE THROMBOCYTOPENIA (ITP)

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Background: Chronic ITP is an autoimmune disorder with persistent thrombocytopenia caused by accelerated platelet destruction and suboptimal platelet production, which in children adversely impacts HRQOL and families. Romiplostim increases platelet production and is approved for treating chronic ITP in adults.

Objectives: This analysis evaluated romiplostim’s effect on HRQOL and on parental disease burden in children with chronic ITP who participated in a randomized, double-blind, placebo-controlled phase 1/2 study.

Design/Method: Children with chronic ITP and persistent severe thrombocytopenia (mean of 2 platelet counts ≤30 x 10^9/L) were stratified by age and randomized (3:1) to romiplostim or placebo once-weekly by subcutaneous injection for 12 weeks. Romiplostim was initiated at 1μg/kg, and adjusted in 2-μg/kg increments every 2 weeks based on platelet counts (maximum dose: 10μg/kg). The Kids’ ITP Tool (KIT), a validated 26-item questionnaire comprised of child, parent-proxy, and parent-impact versions, was completed at week 1 (baseline), week 5, and week 13 (end-of-treatment). KIT scores range from 0–100; higher scores indicate better HRQOL, or on the parent-impact version, less disease burden.

Results: Twenty-two children (16 boys, 6 girls; age: 9.5 ± 5.1 years) with median baseline platelet counts of 13 x 10^9/L (range, 2–29 x 10^9/L) were enrolled. Romiplostim produced platelet responses in 15 of 17 children (compared with 0 of 5 children on placebo) and was well tolerated. KIT scores are summarized below.

Conclusion: Results from this study, albeit with small sample sizes, suggest romiplostim reduces parental burden and improves HRQOL in children with ITP compared to placebo.

(POSTER 154)
A SINGLE INSTITUTION EXPERIENCE WITH AWD-RELATED AVWS: DOES IT IMPACT BLEEDING RISK?

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Background: Acquired von Willebrand Syndrome (AvWS) is a well recognized phenomenon in children with congenital heart disease (CHD). The underlying pathophysiology is thought to result from increased clearance of the largest von Willebrand Factor (vWF) multimers due to turbulent forces within the abnormal cardiac anatomy and/or increased cardiac output (1). After surgical correction of the cardiac defect the multimer pattern has been improved, transiently in the majority of patients studied (2).

Aspirin (ASA) therapy is commonly utilized in patients with complex cardiac defects to reduce platelet initiated thromboses which might occlude critical shunts or conduits created by surgery. ASA therapy may confer an increased risk for hemorrhagic events in CHD patients with AvWS relative to those without AvWS.

Objectives: Describe a single institution experience with the spectrum of CHD associated with AvWS and incidence of bleeding complications in co-morbid CHD and AvWS.

Design/Method: A retrospective chart review of NCH records over a six year period identified 11 cardiology patients with AvWS referred to the hematology service.

Laboratory parameters, anti-platelet therapy, and hemorrhagic events with correlated radiographic imaging studies were reviewed.

Results: The spectrum of heart disease in children with AvWS included Aortic Stenosis (1), Patent Ductus Arteriosus (1), hypoplastic left heart syndrome (1), tetralogy of Fallot (4), ventricular septal defect (3), truncus arteriosus (2) and mitral valve regurgitation secondary to rheumatic fever (1). Patients with AvWS had an average age 8 years at diagnosis. Platelet function analysis (PFA) was abnormal in all patients with AvWS. Red cell distribution (RDW) was abnormal in all but two patients. Eighteen bleeding episodes were documented in seven of the eleven patients and included hemorrhagic CVA (7) and pulmonary hemorrhage (1). Thirteen of these bleeding events occurred in four patients on ASA therapy. No bleeding events have occurred since aspirin was discontinued.

Conclusion: Children with CHD and AvWS taking ASA may be at increased risk for bleeding complications. A prospective study is needed to determine which CHD patients are most likely to develop AvWS and should better define the risk/benefit threshold for ASA therapy in this population.

(POSTER 155)
YOUNG ADULT ONSET OF RECURRENT THROMBOEMBOLISM IN A PATIENT WITH CONGENITAL AFIBRINOGENEMIA

Mindy Grunzke, Neil Goldenberg, Linda Jacobson, Chris Bombardier, Marilyn Manco-Johnson
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Background: Congenital Afibrinogenemia (CA) is a rare bleeding disorder where a deficiency in fibrinogen results in recurrent hemorrhage and shock in newborns. Platelet function analysis (PFA) demonstrates decreased platelet fluidity. Age of onset for recurrent thromboembolism is thought to result from increased clearance of the largest von Willebrand Factor (vWF) multimers due to turbulent forces within the abnormal cardiac anatomy and/or increased cardiac output (1). After surgical correction of the cardiac defect the multimer pattern has been improved, transiently in the majority of patients studied (2).

A 35-year-old female with congenital afibrinogenemia (CA) and recurrent pulmonary emboli presented to our institution for further management and evaluation of abdominal pain. A retrospective chart review of NCH records over a six year period identified 11 cardiology patients with AvWS referred to the hematology service. Laboratory parameters, anti-platelet therapy, and hemorrhagic events with correlated radiographic imaging studies were reviewed.

Results: The spectrum of heart disease in children with AvWS included Aortic Stenosis (1), Patent Ductus Arteriosus (1), hypoplastic left heart syndrome (1), tetralogy of Fallot (4), ventricular septal defect (3), truncus arteriosus (2) and mitral valve regurgitation secondary to rheumatic fever (1). Patients with AvWS had an average age 8 years at diagnosis. Platelet function analysis (PFA) was abnormal in all patients with AvWS. Red cell distribution (RDW) was abnormal in all but two patients. Eighteen bleeding episodes were documented in seven of the eleven patients and included hemorrhagic CVA (7) and pulmonary hemorrhage (1). Thirteen of these bleeding events occurred in four patients on ASA therapy. No bleeding events have occurred since aspirin was discontinued.

Conclusion: Children with CHD and AvWS taking ASA may be at increased risk for bleeding complications. A prospective study is needed to determine which CHD patients are most likely to develop AvWS and should better define the risk/benefit threshold for ASA therapy in this population.

(POSTER 155)
YOUNG ADULT ONSET OF RECURRENT THROMBOEMBOLISM IN A PATIENT WITH CONGENITAL AFIBRINOGENEMIA

Mindy Grunzke, Neil Goldenberg, Linda Jacobson, Chris Bombardier, Marilyn Manco-Johnson
The Children’s Hospital, University of Colorado Denver, Aurora, Colorado, United States

Background: Congenital Afibrinogenemia (CA) is a rare bleeding disorder where a thrombotic association is suspected but unproven. We have followed since childhood a female with CA and spontaneous bleeding who at age 22y suffered recurrent bilateral pulmonary emboli with infarction, and deep and superficial vein thromboses.

Objectives: To describe the clinical course of a CA patient with clinical and research laboratory findings. We hypothesized that the post-pubertal thrombotic complications
OBJECTIVES: To assess whether disparities exist amongst races and to determine the pediatric ITP exist.

Estimated incidence of 5/100,000; however, it is unknown whether racial disparities in

Design/Method: New York Medical College, Valhalla, New York, United States

THROMBOCYTOPENIC PURPURA

RACIAL DISPARITIES IN PEDIATRIC IMMUNE

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New York Medical College, Valhalla, New York, United States

Background: Pediatric immune thrombocytopenic purpura (ITP) occurs with an estimated incidence of 5/100,000; however, it is unknown whether racial disparities in pediatric ITP exist.

OBJECTIVES: To assess whether disparities exist amongst races and to determine the presenting features of ITP in black, white, Hispanic, and Asian patients.

Design/Method: Retrospective chart review of 630 pediatric ITP patients evaluated at New York Medical College from 1985 to 2009. Race was determined by review of medical records, physician recollection and by telephone contact. Observed rates of ITP were compared to expected rates given the incidence of ITP and the area’s demographic construct. Census of children younger than 19 years residing in the Hudson Valley was obtained from the United States Census Bureau. We used a χ2 “goodness-of-fit” test to determine whether the sample distribution differed from the population distribution.

RESULTS: Racial demographics were available for 530 of 630 (84%) patients. The estimated racial demographics of children younger than 19 years residing in the Hudson Valley are as follows: total population, 468,550; 75% Caucasian; 10% Hispanic 10% black; and 5% Asian. Of the 530 identified, 406 (76.6%) were found to be Caucasian, 55 (10%) Hispanic, 36 (7.5%) Black, and 33 (6.2%) Asian. If ITP is detected equally amongst races, one would expect to find 397.5 White patients (observed expected difference [O–E] 8.5 with a residual of 0.426), 53 Hispanic (O - E 2 with a residual of 0.275), 53 black patients (O - E -17 with a residual of -2.335), and 26 Asian (O - E -6.5 with a residual of 1.263). The distribution of our sample over race groups compared with the distribution of the population over race groups had a p-value of 0.063 with the largest contribution due to under-representation of black children in our sample. Of the 36 black children with ITP 18 were found to present with bruising/petechiae, 4 presented with a combination of wet purpura and bruising/petechiae, 3 presented with wet purpura alone, and 11 were asymptomatic.

CONCLUSION: The incidence of ITP in black children may be less than in Caucasian, Hispanic, and Asian children.

STP Funding: NHF/Baxter Clinical Fellowship Award

(POSTER 156)

RACIAL DISPARITIES IN PEDIATRIC IMMUNE THROMBOCYTOPENIC PURPURA

(POSTER 157)

SUCCESSFUL LIVER TRANSPLANTATION TO CORRECT SEVERE CONGENITAL FACTOR V DEFICIENCY IN A CHILD WITH MULTIPLE INTRACRANIAL HEMORRHAGES

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Background: Congenital factor V deficiency is a rare disorder with a prevalence of one per million in the population. Severe deficiency has been associated with a hemophilia-like phenotype including, in rare cases, intracranial hemorrhage in infants. Treatment relies on factor V replacement with fresh frozen plasma (FFP) and platelet transfusions. The long-term complications of chronic FFP exposure can include allergic reactions, thromboembolic events, acute lung injury, and alloimmunization against its components, including factor V. Patients with severe phenotypes who develop these complications may have particularly poor prognoses and no alternative therapies are widely available. Though liver transplantation has been previously shown to correct other factor deficiencies, no reports exist regarding its use in factor V deficiency.

OBJECTIVES: We report successful orthotopic liver transplantation with correction of factor V deficiency in an infant with multiple spontaneous intracranial hemorrhages.

Design/Method: Case report

RESULTS: An infant with severe congenital factor V deficiency (< 1% activity) presented with large bilateral cephhalohematomas at birth. The hematoma resolved with three weeks of daily FFP infusions. At three months of age he developed unilateral epidural, subdural, and intraparenchymal hemorrhages with subfalcal herniation and obstructive hydrocephalus. Bleeding was controlled during and after emergent craniotomy with platelet and FFP transfusions and recombinant factor VIIa. One month later he developed spontaneous bilateral subdural hematomas. FFP infusions were given daily as treatment and then every other day as prophylaxis until a suitable donor was identified and orthotopic liver transplantation was performed. The factor V level increased to 53% immediately post-operative and has remained >80% for the ten months following the procedure. The patient has not experienced further bleeding episodes or complications of the graft and his development is progressing normally.

CONCLUSION: Congenital factor V deficiency can be associated with severe hemorrhagic complications and chronic transfusion therapy may not be tolerated or feasible in all cases. Orthotopic liver transplantation may be considered as an alternative therapy in difficult cases where the morbidities of medical treatment are considered unacceptable. This may especially apply to pediatric cases where the child with severe hemorrhagic complications would require lifelong transfusion therapy.
A retrospective chart review was performed at Mayo Eugenio Litta Children's Hospital, Mayo Clinic, Rochester, MN, using ICD-9 codes for stroke or cerebrovascular accident and all consecutive local and referred pediatric cases < 18 years of age seen between 1981-2006 were identified. Neonates and children with sickle cell disease were excluded.

Results: Hundred and eight patients with 61% males were eligible for this study. Median age was 7 year (range: 1 month–18 years) and median follow-up was 3.7 years (quartiles 25th & 75th: 299 days, 8 years). Table 1 shows the various clinical and radiologic characteristics of our cohort.

Conclusion: Our study represents the largest single institution cohort of childhood AIS. Although several of our findings are in conjunction with the previously published reports, the rate of recurrent stroke is lower in our series. This probably is due to an increase use of acute treatment with anticoagulation and antiplatelet therapy. Despite an extensive follow up, a substantial attrition in our series merits further prospective studies to confirm our findings.

The authors wish to acknowledge Dr. R. Kalpathi for his review of the abstract.

Background: HEMOPHILIA (DOSE)

Objectives: To compare physician-prescribed and patient/caregiver-reported rFVIIa treatment of home bleeds in children (age < 18) and adults.

Design/Method: Fifty-two frequently bleeding CHwI patients (> 4 bleeds over 3 months), prescribed rFVIIa as first-line therapy for acute bleeds, were enrolled in a 3-6 month daily diary study including hourly records of acute bleed treatment.

Results: Prescribed initial rFVIIa dosing for 25 pediatric patients for joint bleeds included median (range) of 200 (61–270) mcg/kg with 56% > = 160 mcg/kg and 40% > = 240 mcg/kg; for 25 adults, 123(81-289) mcg/kg with 48% > = 160 mcg/kg and 32% > = 240 mcg/kg. Prescribed additional dosing in children was 90 (61-270) mcg/kg with 28% > = 160 mcg/kg and in adults 90 (70-270) mcg/kg with 9% > = 160 mcg/kg. Prescribed dosing was similar for muscle and other bleeds. Actual initial dose for children (109 bleeds, 70 hemorrhages) was 211 (74-400) mcg/kg with 63% > = 160 mcg/kg and 29.4% > = 240 mcg/kg; for adults (49 bleeds, 36 hemorrhages) initial dose was slightly higher at 231(59-380) mcg/kg with 67% > = 160 mcg/kg and 43% > = 240 mcg/kg. Median total dose (mcg/kg) per bleeding episode was higher (p = 0.042) for children (900 [74-9601]) than adults (462 [107-21257]). The average of all doses per bleeding episode for children (126 [74-400]) was lower (p = 0.018) than for adults (163 [40-380]) but the number of infusions higher (p = 0.011) for children (6.5(1-105), 30% < = 4 injections) than adults (3.0(1-106), 76% < = 4 injections). On demand treatment of joint bleeds showed more significant age-related differences for initial dose (p = 0.002), total dose (p < 0.001), mean dose (p = 0.003) and number of doses (p = 0.002). There were no thromboembolic events.

Conclusion: Prescribed/actual initial dosing was bimodally distributed around 90 and 200-270 mcg/kg, with approximately 2/3 of children/adults reporting higher initial doses. Actual additional doses were higher than prescribed. Children received higher total doses due to increased infusions per bleed.

Study supported by Novo Nordisk

VENOUS THROMBOEMBOLISM IN PEDIATRIC PATIENTS: EPIDEMIOLOGIC DATA FROM A PEDIATRIC TERTIARY CARE CENTER

Background: Venous thrombosis is an infrequent but serious cause of hospitalization in pediatric tertiary care.

Objectives: To define the epidemiology, incidence and risk factors of serious venous thromboembolism in a pediatric tertiary care center.

Design/Method: Patients discharged from a pediatric tertiary care center over 3 years with ICD9 codes 433.x, 452.x, 415.x, 671.5, and 12.51 were identified. Of 41,906 hospitalizations, 89 met criteria for review. Records revealed that 11 patients (12%) were miscoded, leaving 78 patients for study. The clinical presentation, location of thrombosis, associated risk factors, family history, laboratory evaluation, treatment, and outcome were determined.

Results: The incidence of thrombosis was 18.6 per 10,000 admissions (0.18%) with an equal male/female ratio. Venous thrombosis was more common in Caucasians (ratio of 2:1 with African Americans) compared to the hospital population (1.5:1) (p < 0.05). Locations of thrombosis included deep veins (54%), pulmonary (24%), renal vein (9%), intrahepatic (10%), and intracranial (3%). Significant risk factors for thrombosis included central venous line (29%), malignancy (19%), osteomyelitis/septic arthritis (9%), sepsis (9%), neurologic disability (8%), nephrotic syndrome (5%), and autoimmune disorders (5%). No risk factors were identified in 11% of patients. Six patients (7%) had an inherited thrombophilia including antithrombin deficiency (n = 1), antiphospholipid syndrome (n = 1), factor V Leiden (n = 2), prothrombin mutation (n = 1), and methylenetetrahydrofolate reductase mutation (n = 1). A positive family history of thrombosis was found in 67% of patients with thrombophilia compared to 6% in patients with negative thrombophilia screens (p < 0.005). Treatment included enoxaparin (n = 48), warfarin (n = 11), heparin (n = 7), tissue plasminogen activator (n = 5), argatroban (n = 1), thrombectomy (n = 2), IVC filter (n = 1), and no treatment (n = 15). Outcome data were available for 64% of patients. Of those, 70% demonstrated resolution of the thrombus with no complications. 18% had persistent thrombus, and 12% were deceased. Causes of death were malignancy and congenital heart disease.

Conclusion: Few children manifest inherited thrombophilic defects. In our population, Caucasian race was a positive risk factor. A positive family history predicted thrombophilia. Further research is needed to guide diagnosis and treatment.
(POSTER 162) INTEGRATING COMBINATORIAL CYP2C9 AND VKORC1 GENOTYPES INTO CLINICAL WARFARIN MANAGEMENT IN PUERTO RICAN CHILDREN WITH THROMBOSES: AN ILLUSTRATIVE CASES STUDY

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Background: Warfarin (Coumadin) is an oral anticoagulant that is used to treat and prevent thrombotic events. Warfarin requires careful clinical management to decrease the risk of bleeding during anticoagulation. Growing evidence indicates that up to 60% of the individual pharmacological response to warfarin might be due to genetics and demographic variables (age, weight, height) and affected by polymorphisms in the genes encoding two enzymes, namely, vitamin K epoxide reductase (VKOR) and cytochrome P450 CYP2C9 (1). These polymorphisms have been found to influence warfarin dosing resulting in lower maintenance doses.

Objectives: To identify CYP2C9 and VKORC1 genetic polymorphisms in our pediatric population with thrombotic disease on anticoagulation. To assess for alterations in therapeutic warfarin dosing and any bleeding manifestations in patients with CYP2C9 and VKORC1 polymorphisms.

Design/Method: A study of cases was performed with 20 pediatric patients aged 2 to 18 years with history of venous thrombotic events treated with warfarin in our Pediatric Anticoagulation Clinics at the University Pediatric Hospital in San Juan, Puerto Rico between the years 2004-2008. After a careful record review and physical examination, blood samples were drawn for DNA isolation using the QiAamp DNA Midi Kit (QIAGEN, Valencia, CA) followed by DNA quantification using NanoDrop 2000 technology (Thermo Fisher’s Scientifics, Wilmington, DE). The isolated DNA samples were sent to the CLIA-certified Laboratory of Personalized Health in Genomas, Inc. (Hartford, CT) for DNA typing of 12 major CYP2C9 and VKORC1 polymorphisms using the HILOMet system on LumineX100 x-MAPTM multiplex technology (LumineX Corp., Austin, TX).

Results: CYP2C9 and VKORC1 genetic polymorphisms were found in 80% (16 out of 20) of our Puerto Rican pediatric patients. One patient with history of elevated INR levels presented with both homozygosity for VKORC1 (A/A) and heterozygosity for CYP2C9 (1/3*). Genetic testing of two patients with history of bleeding manifestations (menorrhagia and hematochezia) revealed heterozygosity for VKORC1 (G/A) and CYP2C9 (1/1*), respectively.

Conclusion: CYP2C9 and VKORC1 polymorphism analysis should be considered in pediatric patients with suboptimal INR levels or bleeding manifestations during anticoagulation therapy in order to safely tailor their treatment.


(POSTER 163) ACQUIRED FACTOR IX INHIBITOR AND SUBSEQUENT RESOLUTION WITH IMMUNOTHERAPY IN A CHILD WITH ACQUIRED IMMUNODEFICIENCY SYNDROME

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Background: The development of inhibitors against coagulation factors is rare within the general population and is reported even more infrequently among pediatric patients. Acquired inhibitors against Factor IX occur almost exclusively within the hemophilia B population.

Objectives: We present an isolated pediatric case involving a 17 month old female with Acquired Immunodeficiency Syndrome (AIDS) and no history of congenital hemophilia B population.

Design/Method: We present an isolated pediatric case involving a 17 month old female with Acquired Immunodeficiency Syndrome (AIDS) and no history of congenital hemophilia B population.

Results: Three months post therapy for B-cell Non-Hodgkin’s Lymphoma, she acutely developed a hemorrhagic episode while recovering from a respiratory infection. Initial management included fresh frozen plasma without resolution of the bleeding. Reombinant factor VIIIa was added until hemostasis was achieved. Investigation of the bleeding episode revealed a prolonged activated partial thromboplastin time (aPTT) [64.5 sec with normal range 25–35] and a factor IX level of 4% [47-104%] due to the spontaneous development of a weak inhibitor to factor IX (0.07 Bethesda units). The patient was then treated with intravenous immunoglobulin (IVIG) at 1g/kg/day × 2 days and prednisone (2 mg/kg/day) once daily for seven days followed by a one week taper. Seven days post immunotherapy there was complete resolution of the acquired factor IX inhibitor with normalization of aPTT and factor IX levels.

Conclusion: No other cases have been reported involving acquired factor IX inhibitors within the pediatric HIV/AIDS population. It is our experience that the regimen of IVIG and steroids was both successful and safe in the resolution of this rare coagulopathy without further compromising her immunological status. Further studies are warranted to establish a medical standard of care for these patients.

(POSTER 164) SAFETY OF BeneFIX® (NONACOG ALFA, RECOMBINANT HUMAN FACTOR IX) FOR USUAL USE IN PEDIATRIC PATIENTS: RESULTS FROM A PROSPECTIVE REGISTRY OF EUROPEAN HEMOPHILIA B PATIENTS

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Background: Patients with hemophilia B are treated with factor IX (FIX) replacement therapy. BeneFIX is a currently available recombinant source of FIX. Since a large proportion of hemophilia B patients are in the pediatric age group, it is important to demonstrate the long-term safety of FIX replacement products in this population.

Objectives: To evaluate the long-term safety of BeneFIX in pediatric hemophilia B patients during usual use.

Design/Method: An open-label, multicenter, prospective registry was conducted in 9 European countries to evaluate the safety of BeneFIX in patients with hemophilia B in a usual-use setting. The registry was initiated in March 2002, with a period of enrollment of 7 years. Patients had received at least 1 dose of the original formulation of BeneFIX or a newer, reformulated version of the product. Of 218 patients enrolled in the registry, 66 (30.3%) were pediatric patients < 18 years of age.

Results: A total of 41 (62.1%) of the patients completed the registry per protocol. Seven events of special interest (ESIs) occurred in 6 (9%) patients. Allergic reaction was the sole ESI in 2 patients, lack of drug effect/low FIX recovery occurred in 3 patients, and 1 patient had an allergic reaction and developed a factor IX inhibitor. Two (3.0%) patients withdrew from the registry due to an ESI and/or a serious adverse event. Both were patients who developed an allergic reaction and one was the patient who also developed an inhibitor.

Conclusion: The events reported for pediatric patients participating in this long-term prospective study demonstrated an acceptable safety profile for BeneFIX in the pediatric population. The events did not differ in type, frequency, or severity from those previously observed in pediatric hemophilia B patients treated with BeneFIX.

(POSTER 165) A NOVEL, CELL-SPECIFIC MECHANISM OF NOTCH SIGNALING IN ACUTE LYMPHBLASTIC LEUKEMIA—PARP1/HESI RATIOS

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UT M. D. Anderson Cancer Center, Houston, Texas, United States

Background: Notch signaling plays both oncogenic and tumor suppressor roles in a wide variety of malignancies, depending on cell type. In T cell acute lymphoblastic leukemia (T-ALL), constitutive Notch activation occurs in 50-70% of patients. In contrast, Notch activation in precursor B-ALL leads to growth arrest and apoptosis. The common Notch target gene HES1 contributes to T-cell leukemogenesis, yet can reproduce a tumor suppressor phenotype in pre-B-ALL cells. Therefore HES1 appears to be important to the cell-specific consequences of Notch signaling in leukemia. Greater understanding of the HES1-dependent mechanisms will facilitate therapeutic targeting of Notch in both T-ALL and B-ALL.

Objectives: To determine the cell-specific mechanism of Notch/HES1-mediated growth arrest/apoptosis in pre-B-ALL versus T-ALL.

Design/Method: HES1 transcriptional complex isolation (size exclusion chromatography, immunoprecipitation, mass spec peptide sequencing, co-immunoprecipitation), functional evaluation (chromatin immunoprecipitation PCR, luciferase reporter, PAR ELISAs, ATP assays, subcellular localization, westerns), HES1 domain-specific mutants, 206 pediatric ALL samples run on Human Genome U133A expression arrays, peptide synthesis, viability and apoptosis assays.

Results: We report the novel interaction of HES1 with the apoptosis-related protein PARP1. This HES1/PARP1 interaction leads to de-repression of HES1 target genes and results in striking activation of PARP1, resulting in PARylation of cellular proteins, ATP depletion, nuclear translocation of apoptosis-inducing factor (AIF), as well as caspase-3 cleavage. Importantly, this mechanism occurs only in pre-B-ALL, not T-ALL cells.

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Evaluation of 206 pediatric ALL samples (172 pre-B-ALL, 34 T-ALL) on U133A expression arrays reveals differential Notch pathway gene expression in cytogenetic subgroups. For example, TCF3-PBX1 patients have relatively high PARP1 expression, while T-ALL patients have low PARP1 expression. In contrast, T-ALL patients have uniformly high HES1 expression, and pre-B-ALL patients have low HES1 expression, resulting in striking differences in PARP1:HES1 ratios.

To exploit these differences, Notch ligand-mimicking peptides can induce this HES1/PARPI-mediated pro-apoptotic mechanism selectively in pre-B-ALL, demonstrating a clinically-feasible therapeutic approach.

Conclusion: These data reveal a novel interaction of HES1 and PARP1 in pre-B-ALL which modulates the function of the HES1 transcriptional complex and signals through PARP1 to induce apoptosis. This novel tumor suppressor mechanism involving a Notch-driven, cell-type specific pro-apoptotic pathway, supports the development of Notch agonist-based ALL therapeutics.

**ABSENCE OF BIALLELIC TCR-δ (Loh, Blood, 2009)**

**INDUCTION FAILURE IN PEDIATRIC T-ALL**

**OBJECTIVES:** Patients who fail induction are currently indistinguishable from the majority of patients at diagnosis, and the purpose of this study was to identify them based on DNA copy number analysis of T-ALL lymphoblast specimens obtained at diagnosis, a time when major treatment changes can be instituted.

**DESIGN/METHOD:** Array comparative genomic hybridization (CGH) was performed on genomic DNA extracted from diagnostic specimens from 47 children with T-ALL treated on Children’s Oncology Group (COG) study P9404 or Dana-Farber Cancer Institute (DFCI) study 00-01. Cases analyzed included all of the treatment failure cases available, comprising 9 induction failure and 13 relapse cases, along with 25 matched long-term survivors. TCR-δ status was then confirmed in an independent cohort by quantitative DNA PCR, an assay that is well-suited for clinical application.

**RESULTS:** Several deletions predicted a favorable response to induction chemotherapy, including deletions at T cell receptor gene loci, indicating that V(D)J recombination had occurred. The absence of biallelic TCR-δ locus deletion (ABGD) marks very early T-cell progenitors with germline TCR-δ loci, and was a robust predictor of induction failure (P = 0.0014) as well as inferior event-free and overall survival (P = 0.002 and 0.0007, respectively). A rapid progression in deletions including an abnormal DNA PCR assay was developed to detect the ABGD cases, and this assay was used to validate ABGD as a predictor of patients who fail induction in an independent series of cases.

**CONCLUSION:** Leukemic blasts from children with T-ALL should be evaluated at diagnosis for TCR-δ locus deletion and the ABGD subset, who have a very high probability of induction failure with current therapy, should be assigned to intensified induction therapy in the context of a prospective clinical trial.

**MECHANISM OF ETOPOSIDE ACTIVITY IN TREATING MURINE HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS**

**Theodore Johnson, Catherine Terrell, Michael Jordan**

**Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio, United States**

**BACKGROUND:** The clinical and laboratory manifestations of hemophagocytic lymphohistiocytosis (HLH) are recapitulated in perforin-deficient (prf-/-) mice infected with lymphocytic choriomeningitis virus (LCMV). In this animal model, ineffective cytotoxic T effector cells fail to down-modulate stimulatory signals provided by antigen-presenting cells (APCs). Excessive T cell stimulation leads to mass cytokine production which drives systemic macrophage activation. Thus, at least three critical events occur in the pathogenesis of HLH: 1)Abnormal increase in antigen presentation, 2)Abnormal increase in CD8 T cell activation and cytokine secretion, and 3)Pathological macrophage activation.

**OBJECTIVES:** Determine the mechanism of etoposide activity in treating murine hemophagocytic lymphohistiocytosis.

**DESIGN/METHOD:** Five days after perforin-deficient (prf-/-) mice were infected with LCMV, they were given intraperitoneal injections of either etoposide, dexamethasone, or irrelevant carrier controls. Outcome measures included serial measurements of disease severity, serum interferon-gamma (IFN-g) levels, hemoglobin levels 15 days post-infection, and flow cytometric analyses of whole spleen 8 days post-infection to assess total cellularity, T cell activation, and macrophage infiltration.

**RESULTS:** Etoposide was an effective single agent, whereas dexamethasone was not, in our murine model of HLH leading to significant improvements in disease severity (p < 0.03, etoposide v. control), survival (p < 0.02), peak serum IFN-g levels (p < 0.009), and hemoglobin levels (p < 0.002). Flow cytometric analyses of whole spleen from etoposide-treated animals showed decreases in total cellularity (p < 0.0002, etoposide v. control) as well as absolute numbers of CD8 T cells (p < 0.0002), virus-specific CD8 T cells (p < 0.0002), and macrophages (p < 0.0007). The decrease in T cell numbers was not caused by a direct effect on antigen presentation by APCs, and there was no effect of drug treatment on IFN-g mediated macrophage-dependent pathology in the absence of viral infection.

**CONCLUSION:** Our studies indicate that etoposide acts via cytolytic effects on dividing T cells. This leads to a diminished pool of activated but ineffective responding T cells and attenuation of hypercytokinemia, resulting in decreased tissue infiltration of activated macrophages with less hemophagocytosis. Thus, improvement in HLH-like disease severity and survivability after treatment with etoposide is a direct effect of deleting activated IFN-g producing T cells.

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a rare and usually fatal hyperinflammatory disorder associated with genetic defects of immune function. Survival with current standard HLH therapy, consisting of cytotoxic chemotherapy (etoposide) and non-specific immunosuppression (dexamethasone), is approximately 75% at 8 weeks. Our lab has previously shown that perforin-deficient (pft−/−) mice infected with lymphocytic choriomeningitis virus (LCMV) develop a disorder which is essentially identical to human HLH. Ongoing studies using our unique murine HLH model show that etoposide acts primarily by deleting activated interferon-gamma (IFNg)-producing T cells. While beneficial in reducing HLH related immunopathology, this strategy leads to profound iatrogenic marrow and immune suppression, greatly increasing the risk of opportunistic infection. With the explosion of clinically available immunosuppressive agents, there is a critical need to define which sorts of immune suppression offer the most effective yet focused treatment for HLH.

Objectives: In this study, we utilize our animal model of HLH as a preclinical screen to test the effectiveness of T cell-targeted immune suppression using agents that interfere with interleukin-2 production or signal transduction in T cells.

Design/Method: LCMV-infected pft−/− mice were treated with either Rapamycin, Abatacept, anti-CD2 antibody, combinations of these agents, or control carrier. Outcome measures include serial measurements of disease severity, serum interferon-gamma (IFNg) levels, and hemoglobin levels 15 days post-infection.

Results: LCMV-infected pft−/− mice experience exaggerated symptomatology, massive elevations in serum IFNg, and consumptive anemia before becoming moribund. When these mice are treated with the mTOR inhibitor Rapamycin, either alone or in combination with inhibitors of T cell costimulation, such as Abatacept, they show improvements in disease severity, survival, and hemoglobin levels similar to the benefit seen in animals treated with etoposide monotherapy. We will present data showing the effects of these agents on the activation of dendritic cells, T cells, and macrophages in the context of our animal model.

Conclusion: This data validates the use of this murine model of HLH pathogenesis to define the mechanism(s) of current and novel anti-HLH therapeutic agents. We envision using this model in the future to design rational anti-HLH therapy by combining cytolytic, immunosuppressive, and/or selective biological agents that have complementary modes of action.

(PAPER 170) AUGMENTED BERLIN-FRANKFURT-MUENSTER THERAPY IN ADOLESCENTS AND YOUNG ADULTS WITH ACUTE LYMPHOBlastic LEUKEMIA

Michael Ryting, Deborah Thomas, Anna Franklin, Robert Wells, Patrick Zweidler-Mckay, Kurt Schroeder, Cesar Nunez, Elias Jabbour, Stefan Faderl, Farhad Ravandi, Susan O’Brien, Hagop Kantarjian

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Background: In multiple retrospective evaluations, adolescents 14 to 21 years of age with newly diagnosed acute lymphoblastic leukemia (ALL) have improved outcomes if treated on pediatric chemotherapy protocols as opposed to adult regimens. Augmented Berlin-Frankfurt-Muenster (ABFM) therapy is a successful treatment for adolescents with ALL with acceptable toxicities. We initiated a prospective trial of modified ABFM therapy in patients age 12 to 40 with newly diagnosed ALL and lymphoblastic lymphoma (LBL).

Objectives: The primary objective is to assess the feasibility and the effectiveness of pediatric-based therapy (ABFM) in young adult patients. Secondary objectives are to evaluate the prognostic significance of minimal residual disease (MRD) at the end of induction and of consolidation, to prospectively evaluate DNA methylation and prognosis, and to analyze asparaginase activity and anti-asparaginase antibody formation.

Design/Method: This is a phase II, single arm, prospective trial. Adults between the ages of 12 to 40 with newly diagnosed Philadelphia chromosome negative ALL or LBL are eligible. Diagnosis is confirmed by bone marrow aspiration or, in the case of LBL, diagnostic bone marrow biopsy. Patients already started on steroids are eligible. Induction uses a 4 drug regimen: prednisone, daunorubicin, PEG-asparaginase, vincristine and intrathecal cytosine arabinoside (Cytospan). A day 15 bone marrow determines rapidity of response. Patients in remission at the end of induction follow a chemotherapy regimen patterned directly on ABFM treatment as used in high-risk pediatric ALL. Intravenous PEG-asparaginase is used, and intrathecal treatments are reduced.

Results: 51 patients have completed at least induction. The median age is 20 (14-37). 48 obtained morphological remission by day 28 and 43 patients are rapid responders by marrow morphology at day 15. Grade III-IV hepatic toxicity, thrombosis, and allergic reactions are prominent. 5 patients have had acute stroke-like episodes. 8 patients have died, 2 in complete remission. There have been 8 relapses. The median complete remission duration is approximately 57 weeks, and the overall survival at 2 years is approximately 80%.

Conclusion: A pediatric-based regimen, ABFM therapy, effectively induces remission by morphology and by MRD in young adults with ALL. Response appears to be similar to that in high-risk pediatric patients. Toxicity differs, is significant, but is mostly transient.
were employed to analyze protein expression in acute lymphoblastic leukemia cell lines and patient specimens. A blocking antibody for Nodal was introduced to cell lines in culture and viability and cell cycle analyses subsequently performed.

Results: First, RT-PCR analyses of leukemia cell lines show that Nodal expression correlates with clinical risk classification such that ALL bearing the t(4;11) abnormality expresses a higher level of Nodal than standard risk ALL without a recurrent cytogenetic abnormality. A higher level of Nodal is also detected in relapsed T-cell ALL when compared to newly diagnosed T-cell ALL, which, in turn expresses a higher level than standard risk B-precursor ALL. Second, addition of a blocking antibody for Nodal to leukemia cell lines in culture markedly reduces the percentage of cells in S phase, suggesting that blocking Nodal can reduce the proliferation of leukemia cells.

Conclusion: These preliminary data suggest that Nodal may hold prognostic significance and provide a therapeutic target for children with ALL.

(POSTER 173) PATHWAY BASED EVALUATION OF CYTARABINE PHARMACOGENETICS IN CHILDREN WITH ACUTE MYELOID LEUKEMIA

Kasiani Myers, Robert Gerbing, Todd Alonzo, Christine Phillips, Alan Gamis, Gretchen Radloff, John Perentesis, Stella Davies

Cincinnati Children’s Hospital Medical Center, Cincinnati, Ohio, United States

Background: Cytarabine is a key chemotherapeutic agent in the treatment of acute myeloid leukemia (AML).

Objectives: We utilized a pathway based candidate gene approach to select polymorphic variants likely to be critical to cytarabine metabolism, and genotyped 479 children treated for de novo AML on the Children’s Cancer Group (CCG) 2941 and 2961 protocols for these polymorphisms.

Design/Method: All patients received intensively timed induction therapy with IDA-DCTER/DCTER and were randomized to consolidation therapy with Regimen A: IDA-DCTER/DCTER or Regimen B: IDA-FLAG with a higher cytarabine dose (7500mg/m² versus 800mg/m²). Patients with a matched family donor received intensification with allogeneic transplant; all others received a single course of high dose Ara-C.

Results: There were no significant associations between genotypes and clinical characteristics of leukemia, although polymorphism frequencies did differ significantly by race. Five-year overall survival (OS) from study entry differed between genotypes of the nucleoside transporter gene hENT1 polymorphism rs747199 (46 ± 6% in GG cases versus 58 ± 9% in other patients (p = 0.039). This effect was regimen dependent with OS of 54 ± 10% versus 70 ± 13% in Regimen A (p = 0.067) and 49 ± 10% versus 55 ± 14% in Regimen B (p = 0.644) demonstrating differences in outcome secondary to hENT1 rs747199 can be overcome with higher cytarabine dosing in Regimen B. These findings remained significant in a multivariate analysis adjusted for race as shown in the table below.

Conclusion: Nucleoside transporter polymorphisms may play a critical role in modulating cytarabine transport and influence the outcome of therapy for AML in children.

Tables/Charts:

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Minor Allele Frequency</th>
<th>Hazard Ratio</th>
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</thead>
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<tr>
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<td>rs9139</td>
<td>C–T</td>
<td>0.44</td>
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(POSTER 174) ROLE OF REACTIVE OXYGEN SPECIES IN AMRUBICIN’S DECREASED CARDIOTOXICITY AND ITS LEUKEMIA CELL CYTOTOXICITY

Joy Fulbright, Amirali Hamir, Xiaolin Lu, Jaya Chandra

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Background: The use of anthracyclines in pediatric cancer patients has greatly improved survival, but is limited by due to long-term cardiotoxicity. Reactive oxygen species (ROS) generation and subsequent damage to mitochondria is one of the mechanisms of cardiotoxicity. Amrubicin is a synthetic 9-amino anthracycline approved in Japan for the treatment of lung cancer. In animal studies amrubicin has demonstrated decreased cardiotoxicity compared to doxorubicin.

Objectives: To compare the role of ROS in the development of cardiotoxicity with amrubicin and other anthracyclines and to elucidate the role of ROS in the cytotoxic effect of amrubicin on leukemia cells.

Design/Method: Intracellular glutathione levels were measured in rat cardiomyocytes treated for 24 hours with equimolar doses of anthracyclines. We treated NCrSCID mice with doxorubicin, amrubicin or placebo. After treatment superoxide levels were measured in monocytes of mice using flow cytometry and cardiac tissue was evaluated for pathologic lesions. Apoptotic DNA fragmentation in acute myeloid (ML-1) and lymphoid (Jurkat) cell lines was measured utilizing propidium iodide. Using the dose of anthracycline that induced 50% apoptosis we analyzed a role for ROS in promoting apoptosis by pre-treatment of cells with N-acetylcyesteine, an anti-oxidant, and measurement of the percent sub-diploid using PI staining.

Results: Cardiomyocytes treated with amrubicin demonstrated a 3% decrease of glutathione levels compared to controls whereas daunorubicin caused a 15% and doxorubicin a 34% decrease. Average monocyte superoxide levels in post-treatment with amrubicin or placebo were equivalent whereas doxorubicin caused a near doubling of intracellular ROS. 0/4 mice treated with amrubicin show cardiac lesions compared to 2/4 mice treated with doxorubicin. Treatment with amrubicin have a higher survival rate than doxorubicin treated mice. All three anthracyclines caused apoptosis after 24 hours exposure. N-acetylcyesteine was protective in ML-1 but not in Jurkat cells.

Conclusion: These results suggest that amrubicin may be less cardiotoxic as a result of decreased production of ROS. The cytotoxicity of amrubicin is not always dependent on ROS generation, since lowering of ROS had no effect on the cytotoxicity of amrubicin in Jurkat cells but abrogated the effect in ML-1 cells. Further studies will focus on the molecular basis for this difference.

(POSTER 175) SIG-136 (NSC 694501), A UNIQUE DNA MINOR GROOVE INTERSTRAND CROSS-LINKING AGENT SHOWS SIGNIFICANT ACTIVITY AND DRUG COMBINABILITY AGAINST AGGRESSIVE TYPES OF PEDIATRIC LEUKEMIA

Aru Narendran, Aarthi Jayanthan, Anjali Singh, Andrea Incoronato, Sunil Desai, James Whitlock

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Background: Despite recent advances in the treatment of pediatric leukemia, the outcomes for certain subgroups of patients remain unacceptable. Particularly in juvenile myelomonocytic leukemia (JMML) and infant acute lymphoblastic leukemia (IALL) the malignant cells show aggressive growth properties and increased resistance to chemotherapeutic agents. Hence, novel therapeutic approaches are urgently needed to achieve curative therapies in these children.

Pyrrolobenzodiazepine (SIG-136, NSC 694501) is a highly efficient DNA cross-linking agent with a unique mechanism of action compared with other DNA-binding agents.

Objectives: To establish preclinical data to formulate effective clinical studies for the treatment of refractory pediatric leukemia.

Design/Method: Exponentially growing JMML cell were established from blast cells cultured in the presence of mGM-CSF (n = 3). Leukemia and infant leukemia cells and cell lines were obtained from children with ALL and AML (n = 8) and cultured M). After with increasing concentrations of SIG-136 (10−5nM to 10 four days in culture, viable cells were quantitated by Alamar blue assay. Normal bone marrow stromal cells were cultured under identical conditions. The ability of SIG-136 to synergize with a panel of anti-neoplastic agents was tested by established methods. Results: SIG-136 showed significant cytotoxicity against JMML cells(IC50 < 10−5 nM) to 10 four days in culture, viable cells were quantitated by Alamar blue assay. Normal bone marrow stromal cells were cultured under identical conditions. The ability of SIG-136 to synergize with a panel of anti-neoplastic agents was tested by established methods. Results: SIG-136 showed significant cytotoxicity against JMML cells(IC50 < 10−5 nM) to 10 four days in culture, viable cells were quantitated by Alamar blue assay. Normal bone marrow stromal cells were cultured under identical conditions. The ability of SIG-136 to synergize with a panel of anti-neoplastic agents was tested by established methods. Results: SIG-136 showed significant cytotoxicity against JMML cells(IC50 < 10−5 nM) to 10 four days in culture, viable cells were quantitated by Alamar blue assay. Normal bone marrow stromal cells were cultured under identical conditions. The ability of SIG-136 to synergize with a panel of anti-neoplastic agents was tested by established methods.

Conclusion: SIG-136 inhibited the growth of all pediatric leukemia cells tested with increased susceptibility of more rapidly growing cells. The IC50 values were at least 100 fold higher against normal stromal cells suggest the existence of a therapeutic window for this agent and its ability to synergize with anthracyclines supports the development of further studies and effective clinical trials for currently difficult to cure subgroups of pediatric leukemia.

(POSTER 176) SERUM FERRITIN AS AN OUTCOME PREDICTOR IN PATIENTS WITH HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

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Background: Serum ferritin is elevated in the majority of patients with hemophagocytic lymphohistiocytosis (HLH). Ferritin levels are also an independent predictor of outcome in HLH. The results of this study will help in the development of therapeutic strategies for this often lethal disease.

Objectives: To determine the value of serum ferritin levels as an independent predictor of outcome in patients with HLH.

Methods: A retrospective review was performed of patients admitted to the University of California San Francisco from January 1995 to December 2010 who were diagnosed with HLH. Demographic characteristics, diagnosis, treatment, and outcome were reviewed. Ferritin levels were collected from the time of diagnosis and at intervals thereafter. Serum ferritin levels at diagnosis and at 6 months were compared using a t-test. Multiple regression analysis was used to determine whether serum ferritin levels were predictive of outcome independent of other factors.

Results: A total of 101 patients were identified. The mean age at diagnosis was 5.6 years (range 0.1-17.2). The most common diagnoses were malignancy (38%), infection (27%), and idiopathic (19%). The most common treatment was steroid therapy (90%). Seventy-four patients were alive at the time of the study, and 27 patients had died. The median serum ferritin level at diagnosis was 1186 ng/mL (range 38-40000). The median serum ferritin level at 6 months was 1000 ng/mL (range 0-10000). The serum ferritin level at diagnosis was not significantly different between survivors and non-survivors (p = 0.12). The serum ferritin level at 6 months was significantly higher in non-survivors than in survivors (p = 0.03). Multiple regression analysis revealed that serum ferritin level at 6 months was an independent predictor of outcome (p = 0.04).

Conclusion: Serum ferritin levels at 6 months are a significant predictor of outcome in patients with HLH. This finding may have important implications for the development of therapeutic strategies for this disease.
The NSG xenotransplantation treatment model is a robust model for cytarabine. There was also a 10-fold decrease in total AML cell burden in the spleen relapse at 4 weeks post-treatment. In 5/5 mice treated with 60mg/kg cytarabine there was a significant anti-tumor immune effects that may overcome the immune escape of ALL blasts that leads to relapse. Currently, this is being developed into a phase I clinical trial (TACL group). However, our recent research demonstrated that TLR2 receptors are abundantly expressed on pre-B-ALL cell-lines suggesting that TLR2 agonists potentially have better efficacy in generating anti-ALL immunity. Unlike other TLRs functionally active as homomers, TLR2 forms heteromers either with TLR1 or TLR6 to attain specificity.

**Background:** Patients with Hemophagocytic Lymphohistiocytosis (HLH) have markedly elevated levels of serum ferritin, a key diagnostic criteria. Multiple studies have documented the difficulties in diagnosing HLH and the results of therapeutic trials. However, no study has identified any clinical or laboratory data that can be used as a prognostic variable. We have followed the serum ferritin levels of HLH patients during treatment and hypothesized that a rapid decrease of this number correlated with a better outcome.

**Objectives:** To test the hypothesis that ferritin may be a prognostic variable by reviewing several clinical and laboratory parameters in HLH patients for whom sufficient number of ferritin values were available to correlate with rapidity of response to therapy and survival.

**Design/Method:** The records of 48 HLH patients seen at our institution between 1991 and 2007 were reviewed to obtain age at diagnosis, response to therapy at 10 weeks, survival, serum ferritin levels, serial platelet counts, AST, ALT, and LDH. The primary outcome variable was occurrence of death during study interval. A percent change statistic was derived to summarize the ferritin trend. Univariate analyses were performed using logistic regression; variables found to be significant (p < 0.25) by likelihood ratio chi-square test were considered candidates for inclusion in the multivariate logistic regression model. Continuous variables were evaluated for linear scale in the logit. The G-test was used to assess interaction for all possible two-way product terms. Both the Pearson chi-squared and the Hosmer-Lemeshow goodness-of-fit tests were computed for the final models.

**Results:** Multivariate analysis resulted in a parsimonious model with the main effect of percent change in ferritin adjusted for days from diagnosis to minimum ferritin level, maximum ferritin level, year of diagnosis and age at diagnosis. A patient was 17 times more at risk of death if their percent ferritin decrease was less than 50% as compared to those with a 96% or greater decrease in ferritin. Higher maximum ferritin levels in the first 3 weeks also contributed to the odds of death.

**Conclusion:** Serum ferritin levels are helpful in delineating response to HLH therapy as well as being a prognostic variable for outcome.

**Objectives:** Develop an improved chemotheraphy model of human AML in NSG mice to study AML stem cell chemoresistance and test novel therapies.

**Design/Method:** We injected 5-106 human AML cells into sub-lethally irradiated adult NSG mice via tail-vein. After 12 weeks we assessed peripheral blood (PB) engraftment and bone marrow (BM) myeloid lineages. Serum levels in the first 3 weeks also contributed to the odds of death.

**Conclusion:** Serum ferritin levels are helpful in delineating response to HLH therapy as well as being a prognostic variable for outcome.

**Background:** Xenotransplant models are necessary to study new therapies to treat acute myeloid leukemia (AML). We recently showed that the Toll-like receptor (TLR) 9 agonist, CpG ODN, induces significant anti-tumor immune effects that may overcome the immune escape of ALL blasts that leads to relapse. Currently, this is being developed into a phase I clinical trial (TACL group). However, our recent research demonstrated that TLR2 receptors are abundantly expressed on pre-B-ALL cell-lines suggesting that TLR2 agonists potentially have better efficacy in generating anti-ALL immunity. Unlike other TLRs functionally active as homomers, TLR2 forms heteromers either with TLR1 or TLR6 to attain specificity.

**Objectives:** To test the hypothesis that different synthetic TLR2 agonists (Pam3CSK4 = Pam2, TLR2/1; Pam3CSK4 = Pam3) differ in their ability 1) to transduce specific signalling pathways, 2) to induce apoptosis in pre-B-ALL cells, and 3) to augment pre-B-ALL cell immunogenicity.

**Design/Method:** Pre B-ALL cells pre-treated with each TLR2 agonist were compared to untreated samples using the following assays: 1) Signal transduction was studied by detecting phosphorylation of NFkB along with degradation of IKB by flow cytometry and time response curves were correlated with mRNA expression. 2) Apoptosis/necrosis of pre-B-ALL cells was studied by flow cytometric detection of AnnexinV/7AAD. 3) Augmentation of immunogenicity in cell-culture was investigated by measuring induction of co-stimulatory molecules and allogeneic T-cell proliferation.

**Results:** Pam2 was a more rapid and potent inducer of NFkB signalling than Pam3. Induction of NFkB phosphorylation by Pam2 partially correlated with expression of TLR1 and TLR6 in pre-B-ALL cell-lines. However, Pam3 induced significant apoptosis, while Pam2 did not. Both TLR2 agonists had similar impact on induction of co-stimulatory molecules (CD40, CD86), while Pam3 augmented immunogenicity of pre-B-ALL cell-lines in T-cell allo-reactivity studies (Pam2 not yet tested).

**Conclusion:** Both TLR2 agonists were able to alter ALL immunogenicity. However, only TLR2 agonist Pam3 (TLR2/1) potently induced apoptosis of pre-B-ALL cells. This suggests that TLR2 agonists, alone or in combination with TLR3 agonists, show promising efficacy in improving cure-rates for relapsed pre-B-ALL. However, further investigations are required.

**Background:** Prolonged febrile neutropenia (FN) remains a common problem in pediatric oncology and often leads to empiric computed tomography (CT) of the sinuses, chest, abdomen and pelvis. Little evidence is available as to the diagnostic utility of CT in this setting.

**Objectives:** As more data emerges regarding the long-term risks of CT in children, we aimed to evaluate the diagnostic utility of CT obtained during prolonged FN in pediatric oncology patients. In addition, we investigated whether the underlying diagnosis, symptomatology at the time of CT scanning and length of FN prior to imaging played a role in positive findings.

**Design/Method:** We performed a retrospective review of all admissions in our oncology patients from January 2004-December 2008 who had daily fevers and neutropenia for 4 or more days prompting an immediate CT evaluation. Eligible patients were further evaluated by chart review as to their symptomatology prior to CT imaging. We performed a retrospective review of all admissions in our oncology patients from January 2004-December 2008 who had daily fevers and neutropenia for 4 or more days prompting an immediate CT evaluation. Eligible patients were further evaluated by chart review as to their symptomatology prior to CT imaging. We performed a retrospective review of all admissions in our oncology patients from January 2004-December 2008 who had daily fevers and neutropenia for 4 or more days prompting an immediate CT evaluation. Eligible patients were further evaluated by chart review as to their symptomatology prior to CT imaging. We performed a retrospective review of all admissions in our oncology patients from January 2004-December 2008 who had daily fevers and neutropenia for 4 or more days prompting an immediate CT evaluation. Eligible patients were further evaluated by chart review as to their symptomatology prior to CT imaging.

**Results:** Forty-six patients had fifty-six unique episodes of prolonged FN that resulted in CT imaging. In addition there were thirty-one subsequent scans in these patients during the same hospitalization for continued FN. Positive findings occurred in 13%, 19% and 30% of initial abdomen, chest and sinus CTs, respectively. Only 6.9% of these CT scans led to a change in management of the patient. All of these were scans of the chest. Occult fungal infection was subsequently proven in 30% of those patients initially scanned. Those with clinically important infections had no statistical difference in days of fever or neutropenia or type of underlying malignancy compared with those without infection. Clinical symptomatology was consistent with tephritis, but less so with chest infiltrates or sinus disease.

**Conclusion:** Treatment alteration rarely results from empiric CT imaging in prolonged FN. We show that a majority of abdominal pathology will be preceded by...
symptoms and that pelvic imaging has no utility without preceding symptoms. Although asymptomatic patients were found to have sinus disease it rarely became clinically significant. Therefore, we recommend limiting initial empiric CT imaging in the asymptomatic patient with prolonged PN to the chest only.

(PAPER 180)
PEDIATRIC NEUTROPENIC CANCER PATIENTS HAVE SIGNIFICANTLY LESS CATHER-RELATED MORBIDITY WITH TOTAL IMPLANTABLE DEVICES

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Background: Three primary central venous catheter (CVC) types are utilized in pediatric cancer patients: totally implantable devices (TID), tunneled externalized catheters (TEC), and peripherally-inserted central catheters (PICC). Limited case series in children with cancer have shown a decrease in catheter-associated infection rates with TIDs as compared to TECs. While age, malignancy type, and transplant status have been correlated with the risk for catheter-related infection, no large multicenter study has compared these risks between all three catheter types or evaluated clinical outcome and hospital utilization.

Objectives: To evaluate clinical outcomes associated with CVC type during neutropenic periods in children with cancer.

Design/Method: Retrospective review was performed of coded data of 1,606 hospitalizations of neutropenic patients with childhood cancer and a CVC collected through the multicenter Pediatric Health Information System. Multivariate analysis to assess significance of catheter type was conducted, controlling for age, ethnicity, gender, malignancy category, and transplant status. Clinical outcomes assessed were incidence of serious bacterial infection (SBI), overall mortality, and the duration of hospital stay, intensive care (ICU) time, and antibiotic usage.

Results: Using patients with TECs (n = 488) as a reference group, patients with TIDs (n = 433) spent significantly less time in the hospital (TID:TEC Ratio = 0.55, 95% CI 0.49-0.60; p < 0.003) and in the ICU (0.82, 95% CI 0.72-0.93; p = 0.002), received antibiotics for fewer days (0.51, 95%CI 0.45-0.57; p < 0.001), and had fewer documented SBI (OR 0.49, 95% CI 0.35-0.70; p < 0.001). Patients with PICCs (n = 269) had shorter hospital stays than those with TECs (PICC:TEC Ratio = 1.64, 95% CI 1.47-1.82, p < 0.001); no significant difference in other outcomes were observed. There was no significant difference in mortality.

Conclusion: In neutropenic pediatric cancer patients, TIDs have the lowest risk of SBI with the shortest duration of hospitalization, ICU time, and antibiotic usage.

(PAPER 181)
AMPLIFICATION OF AML1 DOES NOT IMPACT EARLY EVENT-FREE SURVIVAL (EFS) OR OVERALL SURVIVAL (OS) OF CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) TREATED WITH RISK-DIRECTED CHEMOTHERAPY: A REPORT FROM THE CHILDREN’S ONCOLOGY GROUP (COG)

Nyla Heerema, Elizabeth Raetz, Andrew Carroll, Michael Borowitz, Meenakshi Devidas, Eric Larson, Mignon Loh, Leonard Mattano, Kelly Maloney, Brent Wood, Naomi Winick, Stephen Hunger, William Carroll
Children’s Oncology Group, Arcadia, California, United States

Background: Amplification of the AML1 (RUNX1) locus (amp(AML1)) on chromosome 21 is a recurring abnormality in pediatric ALL, which has been associated with increased age, low WBC count and inferior outcomes. Amp(AML1) can be reliably detected by FISH, using TEL/ETV6/RUNX1 probes.

Objectives: To determine the influence of amp(AML1) on early outcome in current COG trials.

Design/Method: We reviewed the clinical features and outcome of children with B-precursor ALL and amp(AML1) treated on current COG trials from 2003–2009 (median follow up 1.2 years, range: 0.33-3.83 yrs). Amp(AML1) required ≥ 5 copies of AML1, with 4 copies on a single chromosome. Children received a 3- or 4-drug induction based on NCI risk group, with post-induction therapy based on early response and established cytogenetic features. Treatment was not altered for patients with amp(AML1). COG required ETVs/RUNX1 FISH starting June 1, 2007, so ascertainment of amp(AML1) may have been incomplete before this.

Results: 89/5470 (1.6%) cases had amp(AML1). Median age was 9.1 years, median WBC was 4.5 × 10⁹/L and 55.1% were female, a higher proportion than previously reported (34%-48%). While the distribution of day 29 MRD by flow cytometry was different, NCI risk group distribution, 2-year EFS and OS were similar between patients with and without amp(AML1) (Table). Ten events occurred in amp(AML1) patients: 3 induction failures, 3 induction deaths, 4 relapses.

Conclusion: Early EFS and OS with risk-adapted therapy are similar in children with and without amp(AML1); however, longer follow up is needed to determine the full impact of amp(AML1) on event outcome.

1Moorman, Blood, 2007; Attarbaschi, JCO, 2008

(PAPER 182)
THE CLASS IV G-CSF RECEPTOR DRIVES DOSE-DEPENDENT ALTERED GROWTH AND DIFFERENTIATION, WHICH IS ASSOCIATED WITH ENHANCED SRC ACTIVATION

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Background: The G-CSF receptor is critical for the survival, proliferation, and differentiation of granulocytes. Alternative splicing results in a full-length class I isoform and a truncated class IV isoform with loss of 3 of 4 critical tyrosine residues. Overexpression of the class IV receptor has been observed in monosomy 7 bone marrow cells.

Objectives: To identify differences in G-CSF Receptor signaling that explains its role in leukemogenesis.

Design/Method: The promyelocytic NB4 cell line was treated with ATRA to induce cell differentiation. mRNA was harvested and q-RT-PCR was performed using specific primers for the class I and class IV transcripts. IL-3 dependent BaF3 cells were transfected with the cDNAs for human class I or class IV receptors. BaF3 transfectants were then grown in G-CSF, and proliferation was assessed by Trypan Blue counting and MTT assay. Whole cell lysates of BaF3 transfectants were prepared after G-CSF stimulation for western blotting to identify phosphoprotein profiles.

Results: ATRA-treated NB4 cells exhibited an increase in differentiated forms: 92% vs 1% in untreated cells. ATRA-treated cells exhibited a relative decrease in class IV expression with a < 1.6-fold IV/I ratio after 6 days of therapy, while control cells remained at a 1.1 ratio. BaF3-class IV cells demonstrated increased proliferation in comparison to BaF3-class I cells after 6 days of treatment with G-CSF at 100 ng/ml. The BaF3-class IV cells showed a bimodal dose-response growth evaluated by MTT assay. At concentrations ≥ 100 ng/ml, BaF3-class IV cells showed increased growth in comparison to BaF3-class I cells. However, at concentrations < 10 ng/ml, BaF3-class IV cells showed decreased growth. Western blotting of G-CSF-stimulated lysates confirmed that phosphoprotein activation was clearly different in the class IV transfectants. Phospho-Akt, phospho-STAT3, phospho-Jak2, phospho-STAT5, and phospho-Erk1/2 showed decreased activation in the BaF3-class IV cells in comparison to the BaF3-class I cells. However, phospho-Src activation was increased in the class IV cells.

Conclusion: The class IV G-CSF receptor is associated with decreased differentiation, increased proliferation, and altered cell signaling. We hypothesize that the class IV G-CSF receptor may play a role in myeloid leukemogenesis by stimulating Src signaling pathways when G-CSF levels are elevated.

1Sioud, PNAS, 2006.

(PAPER 183)
INSIGHTS INTO IMMUNE SUPPRESSION DURING ALL INDUCTION THERAPY: CAN CD4+ T CELLS SAVE THE DAY?

Patrick Zweidler-McKay, Eric D. Wieder, Cara L. Benjamin, Tae-Kon Kim, Wendy Fang, Sankaranarayanan Kannan, Krishna V. Komanduri
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Tables/Charts:

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>With amp(AML1)</th>
<th>Without amp(AML1)</th>
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<tr>
<td>NCI risk</td>
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<tr>
<td>SR</td>
<td>36.2%</td>
<td>63.9%</td>
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<td>HR</td>
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<tr>
<td>EFS overall (2 year)</td>
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<tr>
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<td>Day 29 MRD&lt;0.1%</td>
<td>60.7%</td>
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</table>
Background: ALL induction therapy is very immunosuppressive, and recent data demonstrate that lymphopenia following induction therapy predicts poor outcome. We wondered whether T cell subset analysis could reveal how the remaining lymphocyte repertoire may contribute to outcome.

Objectives: To characterize the effects of ALL induction therapy on T cell subsets and function and to determine which T cell subsets may be associated with improved outcomes.

Design/Method: Prospective analysis of T cell subsets at diagnosis and post-induction (4-6 weeks) on 20 pediatric ALL patients. Using 8-color flow cytometry for CD4, CD8, CD45RA, CD27 and intracellular cytokines (IL-2, IFNα, IL10, TNFα), we defined CD4 and CD8 T cell subsets using CD45RA and CD27 (Naive +/−; Memory: early +/−, intermediate −/−, late +/−) and their function after Staphylococcal enterotoxin B (SEB) stimulation. Regulatory T cells (Treg) were CD4 +/CD25hi/CD127lo.

Results: We found profound quantitative and functional immune suppression during pediatric ALL induction therapy. Overall lymphocyte counts decreased 40% (2400 vs. 1400/mcL), and nearly half of patients did not recover lymphocyte counts above 1000/mcL. We found that the CD4/CD8 ratio skewed toward CD4 cells (2.3 vs. 2.8), and the effects in CD4 and CD8 subsets differed. For example, the fraction of naive CD8 cells increased significantly, while all subsets of memory CD8 cells decreased (1.3 vs. 2.0-fold, p = 0.01-0.05). In contrast, the naive CD4 cells decreased significantly and the early/intermediate CD4 memory cells increased (0.6 vs. 1.8-fold, p = 0.02-0.03). These memory CD4 + T cells represent the majority of lymphocytes post-induction. Unfortunately, SEB-stimulated expression of all cytokines decreased across both CD4 and CD8 groups (0.3-0.6-fold, p = 0.02-0.09). Finally, we observed a significant increase in Tregs (3.1 to 7.0% of CD4+ fraction, p = 0.004).

Conclusion: We reveal contrasting effects of ALL induction therapy on CD4 vs. CD8 T cell subsets and that early/intermediate memory CD4 T cells become the predominant lymphocyte post-induction. This skewing in the CD4 repertoire, and the relative loss of CD8 T cells, might contribute to ALL outcomes, though loss of inducible cytokine production remains a significant issue.

(POSTER 184) ENHANCED ELIMINATION OF 6-MERCAPTOPURINE OR METHOTREXATE-TREATED ALL CELLS BY NATURAL KILLER LYMPHOCYTES IN VITRO: A POTENTIAL MECHANISM OF ACTION OF MAINTENANCE CHEMOTHERAPY

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Background: Maintenance therapy (MT) is an important component of acute lymphoblastic leukemia (ALL) therapy. The operational mechanisms of MT are not completely understood. We hypothesize that apart from direct cytotoxicity, chemotherapy given during MT alters surviving lymphoblasts rendering them susceptible to innate immune response, likely through expression of stress proteins such as NKG2D ligands, specific co-stimulatory or adhesion molecules.

Objectives: The role of 6-mercaptopurine or methotrexate treatment of ALL cells in their elimination by natural killer (NK) lymphocytes and if stress related lymphoblast changes mediate NK lymphocyte reaction against ALL cells were investigated in this study.

Design/Method: Allogeneic NK cell-mediated elimination of REH (TEL/AML-positive) and Raji (mature B cell ALL) cells treated with essential MT drugs was studied. Natural killer cells from healthy volunteers were isolated using MACS technology. After purity evaluation, NK cells were incubated in interleukin-15 overnight. Leukemia cells were incubated in sub-lethal concentrations of 6-mercaptopurine and methotrexate for forty-eight hours. The ALL cells were then co-incubated with NK cells at different ratios. The NK cell-mediated leukemia cell cytotoxicity was measured by flow cytometric cell-mediated cytotoxicity assay, marking effector cells with lineage-specific monoclonal antibodies and staining target cells with propidium iodide and annexin-V and using microscopes for quantification of apoptotic cells. Surface expression of NKG2D ligand (ULBP 1, 2 and 3, MICA and MICB) expression was studied by flow cytometry.

Results: 6-mercaptopurine treatment of REH cells and methotrexate treatment of Raji cells resulted in enhanced NK cell-mediated elimination when compared to untreated leukemia cells by 21% and 22%, respectively. No increase in the expression of NKG2D ligands on drug treated ALL cells were observed.

Conclusion: These findings suggest that enhanced susceptibility of drug-exposed leukemia cells to innate immune response may be an operational mechanism of MT. This mechanism does not appear to involve NKG2D pathway.

(POSTER 185) CALM DEFICIENCY ALTERS EXPRESSION AND ENDOCYTOSIS OF GROWTH FACTOR RECEPTORS

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Background: Gene rearrangements involving the Clathrin Assembly Lymphoid Myeloid Leukemia (CALM) and MLL or AF10 genes have been identified in aggressive leukemias and lymphomas. Expression of CALM-containing fusion proteins immunolabels murine hematopoietic cells, correlating with leukemogenesis in vivo. Disruption of normal CALM protein function as a result of these translocations likely contributes to transformation, although the precise mechanisms are unknown. The native CALM protein is involved in clathrin-mediated endocytosis (CME); it localizes to endocytic vesicles, interacts with membrane elements and clathrin, and when over- or under-expressed, disrupts endocytosis. To better understand the effects of altered CALM activity, we have investigated mutant fit1 mice that lack CALM expression. These mice have iron deficiency, defective hematopoiesis and ultimately premature death. Here we examine CALM’s role in endocytosis and cellular function using cells derived from fit1 mice.

Objectives: To examine the effect of CALM deficiency on surface receptor expression and endocytosis using mouse embryonic fibroblasts (MEFs) derived from normal and CALM-deficient mice.

Design/Method: Day 14 MEFs were generated from normal, heterozygous, and mutant fit1 embryos. Immortalized MEFs were compared in terms of quantity of cell surface and total cellular receptors (by flow cytometry (FC) and Western blotting), receptor mRNA levels, and rate of endocytosis.

Results: Compared with their normal counterparts, cells lacking full length CALM showed altered cell surface receptor expression: transferrin receptor (TfR) protein levels measured by FC were increased two-fold, while EGF receptor (EGFR) levels were reduced two-fold. These results correlated with increased total cellular TfR protein and mRNA levels and decreased total EGFR protein levels. Whereas the rate of internalization of TfR was similar, the rate of endocytosis of EGFR was significantly reduced in CALM-deficient compared with wildtype cells.

Conclusion: CALM deficiency in fit1-derived cells results in increased TfR surface expression; this may partially explain the fit1 iron deficiency phenotype. The reduced EGFR surface expression seen in fit1 cells may be a compensatory mechanism that counteracts the increased growth factor receptor signaling that is associated with impaired endocytosis. Since CALM haploinsufficiency is a feature of CALM-AF10 and MLL-CALM leukemias, these results suggest that the perturbation of normal growth factor biology may contribute to transformation.

(POSTER 186) STXB2 (MUNC18-2) MUTATIONS IN NORTH AMERICAN PATIENTS WITH FAMILIAL HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS

Judith Johnson, Kelly Huizenga, Diane Kissell, Michael Jordan, Udo ZurStadt, Alexandra Filipovich, Kejian Zhang
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Background: Familial hemophagocytic lymphohistiocytosis (FHL) is an autosomal recessive disorder of widespread accumulation of lymphocytes and mature macrophages, sometimes with hemophagocytosis, primarily involving the spleen, lymph nodes, bone marrow, liver, and cerebral spinal fluid. Several genes are specifically associated with FHL including PRFI (FHL2), UNC13D (FHL3), STX11 (FHL4) and STXB2 (FHL5). Mutations in RAB27A have also been described in cases of FHL associated with Griscelli syndrome 2 as well as in isolated FHL.

Objectives: The purpose of this current study was to determine the frequency of STXB2 mutations in North American patients with presumed FHL.

Design/Method: PCR-based sequencing of the entire coding region and exon/intron boundaries of the STXB2 gene was undertaken on stored DNAs collected from 27 unrelated symptomatic individuals previously referred for evaluation of FHL who were of diverse geographic, racial and ethnic origins.

Results: We identified homozygous mutations in two patients. Heterozygous mutations were identified in three additional patients suggesting that unidentified mutations in STXB2 or interactions between STXB2 and other genes or environmental factors may play a role in STXB2-related FHL.

Conclusion: In conclusion, STXB2 mutation(s) are identified in approximately 20% of North American patients with FHL and sequencing of STXB2 should be included as part of the standard genetic evaluation of these patients.
Detect serious bacterial infections. Procalcitonin (PCT) levels on admission have bacteremia; however, they are not always reliable. Many biomarkers have claimed to discriminate the absence of bacteremia. Serial PCT has a high negative predictive value of 91%. The sensitivity of serial PCT (74%) in this population was affected by the large number of patients (n = 15) with no bacterial growth that had positive PCT levels at both time points.

Conclusion: Negative serial PCT levels showed to be a specific biomarker in discriminating the absence of bacteremia. Serial PCT has a high negative predictive value and in correlation, this may allow for earlier discharge for patients who are clinically well appearing with low ANCs if blood culture show no growth and negative serial PCT.

*(Reitman, Presented at AAP NCE, 2009)*

**HYPERSENSITIVITY REACTIONS TO PEG-ASPARAGINASE IN CHILDREN FOLLOWING INTRAVENOUS ADMINISTRATION**

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Aflac Cancer Center and Blood Disorder Clinic and Emory University, Atlanta, Georgia, United States

Background: PEG-asparaginase (PEG-ASP) is a crucial component of pediatric acute lymphoblastic leukemia (ALL) therapy. Although hypersensitivity reactions to PEG-ASP occur less frequently than with other formulations, they are not uncommon and have an adverse effect on patient outcomes. It is common practice in most pediatric ALL protocols to administer PEG-ASP as an intramuscular (IM) injection. In children, repeated IM injections are uncomfortable and create a significant amount of anxiety for patients and families who already deal with considerable stress during treatment. Preliminary data suggests that IV intravenous (IV) administration of PEG-ASP is safe and results in an appropriate level of asparagine depletion comparable to IM dosing.

Objectives: To review our experience with PEG-ASP administered intravenously and determine the incidence of hypersensitivity reactions.

Design/Method: A retrospective review was performed of pediatric patients that received IV PEG-ASP between January 2006 and May 2008 at a single institution. Results: 41 patients received at least one dose of IV PEG-ASP during the study period. Four of these patients had hypersensitivity reactions (9.8%). No patient experienced a severe (grade 4) hypersensitivity reaction to IV PEG-ASP. Dosing was variable and patients were often receiving other chemotherapy agents in addition to PEG-ASP. Patients that received IV PEG-ASP were often heavily pretreated and 25 patients had received previous treatment that included intramuscular (IM) PEG-ASP. Overall, a total of 119 doses were administered. Most patients received more than one dose of IV PEG-ASP with a median of 2 doses (range: 1–7). Other side effects seen with IV PEG-ASP included 2 episodes of pancreatitis. Thrombosis did not occur in any patient.

Conclusion: In this group of patients, the administration of IV PEG-ASP did not result in an increase in the incidence of hypersensitivity reactions when compared to other pediatric studies involving IM PEG-ASP. Further studies are needed to determine whether the use of IV PEG-ASP is as safe and effective as IM PEG-ASP when utilized in pediatric ALL therapies.

**EXTERNAL VENOUS CATHETERS HAVE A HIGHER RATE OF EARLY REMOVAL THAN SUBCUTANEOUS PORTS IN PEDIATRIC CANCER PATIENTS**

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Primary Children’s Medical Center, University of Utah, Salt Lake City, Utah, United States

Background: Most pediatric cancer patients have either an external venous catheter (EC) or a subcutaneous port (SP) placed for blood draws and medication administration. The type of line placed depends on the diagnosis, expected treatment, patient age, and the preferences of the patients, parents, and physicians. Both types of central lines are prone to complications that can lead to removal prior to the completion of therapy.

Objectives: To compare the rates and causes of early removal of SPs versus ECs in pediatric cancer patients, using a hospital-wide central line database.

Design/Method: This was a single institution, retrospective cohort study of all central lines placed in pediatric cancer patients between January 2000 and December 2009. The association of line type with early removal was calculated by univariate analysis and multivariate logistic regression.

Results: The database included 743 patients with 878 lines (403 SPs, 475 ECs). The mean patient age at SP and EC placement was 9.9 and 6.8 years, respectively (p < 0.001). Of patients receiving SPs, 220 (55%) had acute lymphoblastic leukemia (ALL), 4 (1%) acute myeloid leukemia (AML), and 179 (44%) solid tumors. Of patients receiving ECs, 159 (33%) had ALL, 80 (17%) AML, and 236 (50%) solid tumors. Having an EC increased the odds of early removal (OR 5.2, 95% CI 3.4–8.1) with 130 (27%) ECs and 27 (6.7%) SPs being removed early. The mean age at early removal was 13 and 5.9 years for SPs and ECs, respectively (p < 0.001). Having an EC remained a risk factor independent of age and diagnosis (OR 6.3, CD). The reasons for SP removal were infection (26%), non-functioning line (26%), patient preference (22%), therapy change (15%), damage (7%), and clot (4%). The reasons for EC removal were infection (47%), patient preference (19%), non-functioning line (18%), damage (12%), therapy change (2%), and clot (2%).

Conclusion: External venous catheters carry a higher risk of early removal than subcutaneous ports in pediatric oncology patients. Infection is the most frequent cause of early line removal. This data may be taken into consideration when deciding between these two types of lines.

**DOES fMRI ADD TO A MULTIVARIATE ASSESSMENT OF COGNITIVE OUTCOME IN SURVIVORS OF CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA?**

Shadi Farzighohar, Mary Hughes, Melanie Comito, Claire Flaherty-Craig, Qing Yang, Paul Eslinger

Penn State Hershey Children’s Hospital, Hershey, PA, United States

Background: Neuro-cognitive effects have been described in children with Acute Lymphoblastic Leukemia (ALL), due to their treatment that includes central nervous system (CNS) prophylaxis.

Objectives: To undertake functional brain imaging (fMRI) in children with ALL treated with prophylactic CNS-directed chemotherapy, without whole brain irradiation.

Design/Method: 30 subjects, aged 8-17 years, receiving maintenance ALL therapy or completed therapy were enrolled. The sample comprised 3 cohorts (10 in each cohort), with increasing time since diagnosis. fMRI brain activation patterns in the ALL sample during a working memory task were compared to healthy controls. Participants also underwent standardized cognitive tests.

Results: Cohort I (1-3 years post-diagnosis, still receiving chemotherapy) showed widespread overactivation of multiple brain regions in comparison to controls, although accuracy on the working memory task was significantly lower (see figure). In contrast, Cohort II (3-5 years post-diagnosis, off therapy) generated maps with less activity than controls and also scored lower on neurocognitive tests. Cohort III (>5 years post-diagnosis, off therapy) showed localized overactivation in parietal regions with lower working memory scores than controls.
Conclusion: JMRI showed widespread overactivations for subjects still receiving therapy. This suggests they recruited more widespread neural resources for the cognitive task while still performing more poorly than controls, which may indicate attempts to compensate for neurocognitive difficulties. However, this pattern was variable in its course with increasing time after diagnosis, where we observed a mix of brain underactivation and overactivation. Longitudinal studies with larger samples are needed to confirm and clarify these brain alterations that may underlie cognitive changes in ALL patients.

Tables/Charts:

(POSTER 191)
IMPROVEMENT OF OUTPATIENT ONCOLOGY CLINIC EFFICIENCY UTILIZING STANDARDIZED CLINIC TRACKING DATA

Susan Rheingold, Iris Insogna, Nancy Sacks, Carol Simpson, Leah Commander, Colleen Wallace, Diane Baniewicz, Kimberly Esposito

The Children’s Hospital of Philadelphia, Philadelphia, Pennsylvania, United States

Background: The Oncology Clinic at CHOP sees ~60 patients/day in limited space. As a quality improvement project we analyzed a standard vincristine visit to help identify periods of non-value added time (NVAT) and studied interventions to improve efficiency of outpatient flow.

Objectives: To use a standard oncology visit to study interventions to reduce NVAT and improve flow.

Design/Method: Since 2006 we have captured time-specific data on patients receiving vincristine only (VCR), as defined by physical exam, labs, and drug. Patients did not qualify if they received other intravenous or unexpected interventions. The following time points were tracked:

- Arrival Time
- Triage
- NP/Fellow Visit
- Attending Visit
- VCR activated
- VCR made
- VCR given
- De-accessed “Fast-track” required arrival before 9:30am and prioritization in registration, triage, clinician schedule and pharmacy.

Results: Since 2006, 100 VCR patients have been tracked (48 leukemia/lymphoma, 37 solid tumor, 15 brain tumors). Average total yearly visit time (from triage to de-access) ranged from 92 to 115 minutes, overall averaging 103 minutes (40-200 mins). In our initial 2006 data set, appointments before 10am and after 2 pm were 32 and 45 minutes faster respectively than midday appointments, although not as significant in subsequent cohorts. Patients requiring IV placement had a total visit time 25 minutes shorter than patients requiring central access. Forty-two percent of patients were >15 minutes late, 21% were >15 minutes early and 37% were on time. Total visit time had no correlation to actual arrival time.

Interventions studied to decrease NVAT included a voice activated communication device which improved pharmacy-RN communication; reflected in decreased VCR-made to given time (~10 minutes), but did not alter total time. An improved electronic scheduling system made no difference in compliance with scheduled visit time.

Utilizing historical VCR data we created the VCR “fast-track” in an effort to decrease total visit time and improve appointment-time compliance. Initial fast-track data on 10 patients revealed an improved average visit of 83 minutes (47-124 mins). Concomitant non-fast-track patients had an average visit of 121 minutes (79-170 mins).

Conclusion: Fast-track patients spent significantly less time in clinic, demonstrating successful quality improvement. Utilizing standardized visit time-tracking we can assess ongoing interventions and decrease NVAT.

(POSTER 192)
INCIDENCE AND PREDICTORS OF TREATMENT RELATED MORTALITY IN ACUTE LYMPHOBLASTIC LEUKEMIA IN THREE CENTRAL AMERICAN COUNTRIES

Sumit Gupta, Federico Antillon, Miguel Bonilla, Ligia Fu, Scott Howard, Raul Ribeiro, Lillian Sung

Hospital for Sick Children, Toronto, Ontario, Canada

Background: Cure rates for children with acute lymphoblastic leukemia (ALL) in low-income countries lag behind those in high-income countries (HICs), in part due to higher rates of treatment related mortality (TRM). The characterization and identification of predictors of TRM allows for the rational design of targeted interventions.

Objectives: To describe the incidence, timing and predictors of TRM among children with ALL in El Salvador, Guatemala and Honduras.

Design/Method: We analyzed patients diagnosed with ALL between January 2000 and March 2008, and who started induction chemotherapy. Almost all were treated with the EGS-II protocol, which was adapted from the St. Jude Total XII and XV protocols. Data were collected prospectively by trained data managers. Demographic, disease-related, socioeconomic and nutritional variables were all examined as potential predictors of outcome.

Results: Among 1,670 patients, 859 (51.8%) were classified as high-risk. TRM occurred as a first event in 156 children (9.3%); 92/156 (59.0%) occurred during induction therapy and 37/156 (23.7%) during maintenance therapy. While the TRM rate decreased in those diagnosed after July 1, 2004 (11.2% vs. 7.9%; P = 0.02), the rate of induction death did not change (5.2% vs. 5.8%; P = 0.58). Predictors of induction death included high-risk ALL (odds ratio [OR] = 1.77; 95% confidence interval [CI] 1.44-2.79; P = 0.01), higher initial WBC count (OR per 50 × 10^9/L = 1.10, 95% CI 1.01-1.19; P = 0.02) lower initial platelets (OR per 10 × 10^12/L = 0.94, 95% CI 0.90-0.97; P < 0.0001) and longer travel time to clinic (OR = 1.05, 95% CI 1.01-1.11; P = 0.02). Nutritional variables did not predict induction death. TRM in maintenance was not significantly predicted by any variable examined.

Conclusion: In Central America, TRM remains a significant cause of treatment failure in children with ALL. Unlike in HICs, a significant proportion of TRM occurred during maintenance therapy, though this proportion decreased over time. Interventions targeting emergency care access and education may benefit high-risk patients. Supportive care interventions targeting children who present with high WBC and low platelets may decrease induction death.

(POSTER 193)
THE PROGRESSION OF BONE MINERAL DENSITY ABNORMALITIES POST-CHEMOTHERAPY IN CHILDREN WITH ALL

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Background: As the survival rate for children with Acute Lymphoblastic Leukemia (ALL) in low-income countries lag behind those in high-income countries (HICs), cure rates for children with acute lymphoblastic leukemia (ALL) in low-income countries lag behind those in high-income countries (HICs), yet the progression is poorly understood. Consequently, we must design safer treatment protocols and better treat late side effects. ALL treatment is associated with bone demineralization, yet the progression is poorly understood.

Objectives: To determine the progression of bone demineralization post-chemotherapy in childhood ALL survivors.

Design/Method: We analyzed patients treated with the EGS-II protocol, which was adapted from the St. Jude Total XIII and XV protocols. Data were collected prospectively by trained data managers. Demographic, disease-related, socioeconomic and nutritional variables were all examined as potential predictors of outcome.

Results: Among 1,670 patients, 859 (51.8%) were classified as high-risk. TRM occurred as a first event in 156 children (9.3%); 92/156 (59.0%) occurred during induction therapy and 37/156 (23.7%) during maintenance therapy. While the TRM rate decreased in those diagnosed after July 1, 2004 (11.2% vs. 7.9%; P = 0.02), the rate of induction death did not change (5.2% vs. 5.8%; P = 0.58). Predictors of induction death included high-risk ALL (odds ratio [OR] = 1.77; 95% confidence interval [CI] 1.44-2.79; P = 0.01), higher initial WBC count (OR per 50 × 10^9/L = 1.10, 95% CI 1.01-1.19; P = 0.02) lower initial platelets (OR per 10 × 10^12/L = 0.94, 95% CI 0.90-0.97; P < 0.0001) and longer travel time to clinic (OR = 1.05, 95% CI 1.01-1.11; P = 0.02). Nutritional variables did not predict induction death. TRM in maintenance was not significantly predicted by any variable examined.

Conclusion: In Central America, TRM remains a significant cause of treatment failure in children with ALL. Unlike in HICs, a significant proportion of TRM occurred during maintenance therapy, though this proportion decreased over time. Interventions targeting emergency care access and education may benefit high-risk patients. Supportive care interventions targeting children who present with high WBC and low platelets may decrease induction death.

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increased in 66%. There were no differences between males and females in any age cohort.

Conclusion: Older age at time of treatment is a risk factor for bone mineral loss after ALL treatment, but sex is not a determinant of BMD loss. Z-score continued to decrease in 66% of females after 820 2010 ASPHO ABSTRACTS second cycle of ABVD. IR patients received 6 cycles of ABVD and IFRT (2000 or 2500 cGy) at the end of all chemotherapy, according to RER status after 4 cycles of ABVD. Toxicities were assessed according to CTC version 3.0 criteria. 

Results: From 1/2004 to 08/2009, 169 evaluable (107 IR and 62 LR) patients with a median age of 7.8 years were treated with this regimen. (70%) of the LR patients were RER did not require IFRT, and 60% of IR patients were RER and only required 2000 cGy. With a median follow-up time of 2 years we have not observed any relapses or deaths (100% EFS and OS). Six patients abandoned therapy prior to completion (all IR) and 3 patients (1 LR and 2 IR) were lost to follow up after therapy. There were no grade 4 toxicities.

Conclusion: This regimen was well tolerated and produced excellent results. Abandonment in the higher risk group is still the greatest problem and earlier and more aggressive interventions are needed to target this group. 

(POSTER 194) SUBCUTANEOUS PORTS VERSUS EXTERNAL VENOUS CATHETERS FROM THE PERSPECTIVE OF PEDIATRIC ONCOLOGY PATIENTS AND THEIR FAMILIES

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Background: Pediatric oncology patients and caregivers are often asked to choose between external venous catheters (EC) and subcutaneous ports (SP). Data regarding the patients’ and parents’ perspectives regarding these catheters are scarce.

Objectives: To compare ECs and SPs from the perspective of pediatric oncology patients and their families.

Design/Method: A 22-item survey addressing line complications, factors influencing line choice, and line preference was administered to clinic patients over an 18 month period. Scores were calculated for satisfaction, ease of care and impact on daily living using a scale of 1 to 7 with 1 = “very unsatisfied, very difficult, or very large impact” and 7 = “very satisfied, very easy, and no impact” respectively. Analysis included Fischer’s exact and Wilcoxon Rank-Sum tests.

Results: The study population consisted of 143 patients: 66 had SPs, 47 had ECs, 30 had both. Groups did not differ in age. The most frequently noted factors influencing line choice were a “lower infection risk” and “the ability to swim/bathe” in the SP group and “doctor’s recommendation” and “painless access” in the EC group. EC patients more frequently reported limitations in sports, swimming/bathing, school, and travel (P < 0.01 each). Compared to SPs, ECs had higher rates of infection, clots, damage, malfunction (P < 0.001 each) and negative body image (P < 0.005). Median scores for satisfaction, ease of care and impact on daily life were 7 and 5, 7 and 5, 4.5, 6 and 3 for SPs and ECs, respectively (P < 0.001 each). Of 57 patients utilizing outside institutions for care, 58% of SP patients and 19% of EC patients had concerns about line care at those institutions (P = 0.003). Lines were removed prematurely in 7.3% of SPs and 27% of ECs (P = 0.006). 90% of SP patients and 43% of EC patients would choose their lines again (p = 0.003). 90% of patients with both SPs and ECs, 70% preferred SPs, 13% preferred ECs, and 17% had no preference (P < 0.001).

Conclusion: Patients and families with SPs reported greater satisfaction, fewer limitations and fewer complications than those with ECs. This data may help in counseling families regarding the choice of central lines.
Background: Follicular lymphoma (FL) is a rare and poorly characterized pediatric malignancy which accounts for less than 2% of all pediatric NHL. It is clinicopathologically distinct from adult FL and presents as a high grade but localized tumor commonly affecting the cervical lymph nodes and tonsils. It has a predilection for the male sex and usually responds well to aggressive multiagent chemotherapy.

Objectives: To retrospectively review our experience of using Rituximab based chemotherapy for the management of FL in children.

Design/Method: Retrospective case series.

Results: Six pediatric patients were diagnosed with FL at the Mayo Clinic from 1999–2009. Age at diagnosis ranged from 10–18 years (median 15.5yrs) with a male-to-female ratio of 1:3. Sites involved at presentation included cervical lymph nodes and tonsils (n = 4), parotid gland (n = 1) and duodenum (n = 1). Three patients had stage I, 2 stage II and 1 stage III disease at diagnosis. Five of the 6 patients had grade 3a lymphomas and 1 had grade 1. On immunohistochemistry, all patients expressed BCL-6, CD20 and CD10. Four patients expressed BCL-2. BCL-2 tended to be positive in older patients, but was not related to outcome. BCL-2 gene rearrangement was tested in 2 patients and was positive in both. Four patients underwent complete excision of the tumor, and 2 patients had incomplete excision/biopsy. Two patients received involved field radiation. All patients received Rituximab based chemotherapy. Five patients received R-CHOP, whereas 1 patient was treated with Rituximab monotherapy followed by R-CVP and eventually R-CHOP. All but one patient are in complete remission with a follow up of 9–72 months (median 28mo). No major side effects to chemotherapy were reported.

Conclusion: Adult studies have demonstrated an advantage of adding Rituximab to conventional chemotherapy with significant increase in the remission rate, remission duration and even overall survival for FL patients. We report the first pediatric cohort of FL patients treated with Rituximab based chemotherapy. All patients responded to therapy and 5/6 (83%) are in complete remission. Rituximab based chemotherapy appears to be safe and effective in the management of pediatric FL. Larger multicenter trials are needed to provide therapeutic guidelines.

(PAPER 198) RITUXIMAB BASED CHEMOTHERAPY FOR PEDIATRIC FOLLICULAR LYMPHOMA: A SINGLE INSTITUTION EXPERIENCE

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Background: Congenital rubella syndrome (CRS) is a severe multisystemic disease caused by infection with the human rubella virus during pregnancy. CRS occurs in 1 of 2000 to 1 of 4000 live births and usually results in severe morbidity and death. The two most important sequelae are hearing loss and intellectual disability.

Objectives: The objectives of this study were to evaluate the experience of the CRS clinic at the Mayo Clinic, Rochester, MN, United States over the past 16 years with respect to the management of CRS. The study was also designed to determine the most appropriate multidisciplinary clinic model that could be used in other settings.

Design/Method: This is a retrospective study of all the patients who were seen at the CRS clinic at the Mayo Clinic, Rochester, MN, United States between 2000 and 2016.

Results: A total of 162 patients were seen during this period, with an average of 10 patients seen annually. The most common presenting symptoms were hearing loss, intellectual disability, and ear infections. The majority of patients (85%) had been referred to the Mayo Clinic for evaluation and management. The median age at diagnosis was 1 year (range 0–5 years). The median age at the time of the most recent visit was 22.5 years (range 2–40 years). The median follow-up was 11.5 years (range 0–18 years).

Conclusion: The Mayo CRS clinic has followed a series of patients with CRS for the past 16 years. The Mayo CRS clinic model is successful in providing comprehensive care to patients with CRS and is recommended as a model for all similar centers. Further research is needed to evaluate the long-term outcomes of CRS patients and to identify risk factors for the development of hearing loss and intellectual disability.
Background: Improved survival in childhood acute leukemia has drawn attention to the impact of treatment related febrile neutropenia, and the impact on health care costs and potentially on health related quality of life.

Objectives: To determine nationwide hospital utilization and charges associated with the in-patient treatment of fever and neutropenia in childhood Acute Lymphoblastic Leukemia (ALL) and Acute Myeloid leukemia (AML) in the United States.

Design/Method: National Inpatient Sample (NIS) database is the largest all payer database for hospital inpatient stays in US. We analyzed the data for the year 2007 report by 1044 hospitals located in 40 states and has records of 8 million hospital stays. Using ICD 9 codes we determined the number of discharges for pediatric patients with ALL (204.0) and AML (205.0). In order to exclude patients admitted for in-patient chemotherapy we excluded discharges for ALL, which included a code for the administration of chemotherapy (99.25 and V58.11). Further, for both ALL, and AML we excluded all discharges which included a code suggesting that the patient has had a bone marrow transplantation for both ALL and AML(64, 279.5, 42.81 and V42.82). In this cohort of discharges, we determined the discharges, which also had codes for both fever (ICD 780.6), with or without neutropenia (ICD 288) and analyzed their healthcare utilization and outcome parameters.

Results: There were a total of 25,654 discharges for ALL and 4695 for AML. Healthcare utilization and outcomes are described in table 1. Conclusion: Inpatient febrile neutropenia in childhood ALL and AML is associated with significant length of stay, cost and very low mortality especially in ALL. These data support the need for continued evaluation of outpatient therapies for the management of chemotherapy associated febrile neutropenia in low risk leukemia patients.

Tables/Charts:

(PORTR 202) POTENTIATION OF VINCISTINE TOXICITY WITH CONCOMITANT FLUCONAZOLE PROPHYLAXIS IN CHILDREN WITH ACUTE LYMPHOBlastic LEUKEMIA

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Background: Use of azole antifungals as prophylaxis is becoming an increasingly common practice in acute lymphoblastic leukemia (ALL). Limited literature in adults heightened the awareness of possible increased vincristine (VCR) toxicity in patients receiving concomitant azole therapy. This is due to inhibition of cytochrome P450 3A4 that may increase overall exposure to VCR resulting in dose reductions or omissions.

Objectives: To determine whether the use of fluconazole prophylaxis increases vincristine toxicity in children with ALL.

Design/Method: We retrospectively evaluated children with ALL between January 2004 and December 2009. Patients were subdivided into two groups based on whether or not they received fluconazole prophylaxis during induction therapy. Data was collected for up to three months following the completion of induction therapy. Gastrointestinal (GI) toxicity was defined as documented constipation with modification of bowel regimen and neuropathy was determined using the modified "Baltu" pediatric scale.

Results: Thirty-one patients were included for analysis (N = 16 azole; N = 15 no azole). The median age was 6 years and there were 17 males (55%). Neuropathies were reported more frequently in the azole group compared to no azole group (69% vs. 27%, p = 0.03). Patients in azole group are older compared to no azole group (10.5 years vs. 4 years, p = 0.003). There was no significant difference in gender, race, steroid use (prednisone or dexamethasone), GI toxicity, VCR dose modification and the rate of fungal or bacterial infections between these two groups. Multivariate logistic regression analysis showed only advanced age is an independent predictor of neuropathy (odds ratio of 1.44 (95% CI, 1.01–2.05, p = 0.045)). Patients receiving fluconazole were 4.5 times more likely to experience neuropathy than those not receiving azole, however this was not statistically significant.

Conclusion: We report an increased incidence of VCR toxicity due to concomitant fluconazole prophylaxis in children with ALL which is consistent with the adult study. Lack of significant difference in VCR dose modifications in our study is probably due to a small sample size. Large prospective studies are needed to confirm our findings. Meanwhile judicious use of azole prophylaxis is recommended in this patient population.

(RPOST 203) CHARACTERISTICS AND IMPLICATIONS OF SPINAL EPIDURAL LIPOMATOSIS IN CHILDREN WITH HEMATOLOGIC MALIGNANCIES

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Background: Patients with leukemia or lymphoma require multiple lumbar punctures for diagnosis or therapy. Abnormal fat deposition in the epidural space, or spinal epidural lipomatosis (SEL), may cause obstruction and impede cerebrospinal fluid flow, thus compromising treatment. Risk factors for SEL include glucocorticoids (exogenous or endogenous) and obesity. Despite frequent treatment with steroids, little is known about SEL in patients with hematologic malignancies.

Objectives: We estimate the frequency, identify potential risk factors, and determine the clinical consequences of SEL in patients with leukemia or lymphoma.

Design/Method: Patients were identified through a query of the radiology electronic database for "lipomatosis" at St. Jude Children’s Research Hospital from 1999-2009. Medical records and MRI images were reviewed retrospectively.

Results: Among the 1170 patients with acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML) or non-hodgkin lymphoma (NHL), 13 (8 ALL, 1 AML, 4 NHL) were diagnosed with SEL (incidence 1.1%). The median age was 11 years (range 3-22); 5 patients were female and 8 male. All patients had at least one abnormal value on lipid profile and 8 were overweight or obese. Nine cases were diagnosed by MRI after unsuccessful lumbar puncture within one month (range 8 to 30 days) of their primary diagnosis. Additional indications for SEL leading to SEL diagnosis were lower back pain in 2 patients, and lower extremity weakness associated with sepsis and draining gluteal cleft in one case each. Prior to SEL diagnosis, all patients had received triple intrathecal therapy that included hydrocortisone and 12 patients also received systemic glucocorticoids. Ommaya reservoir placement was necessary for 4 patients, 3 of whom had more than 30% obstruction of the lumbar spinal canal. No patient developed progression of SEL with continued therapy. Eleven patients are in remission for a median of 2.1 years (range 0.5-7.6 years). One patient with AML developed bone marrow relapse and another with NHL developed CNS relapse; both patients subsequently died of progressive malignancy.

Conclusion: SEL is a rare but important diagnosis to consider in leukemia/lymphoma patients for whom lumbar puncture is unsuccessful. Placement of an Ommaya reservoir may safely facilitate CNS-directed therapy in severely affected patients.

(PORTR 204) INDUCTION OF AUTOPHAGY BY SPHINGOSINE KINASE INHIBITOR

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Background: Autophagy is a cellular pathway involved in bulk protein and organelle degradation. Although it is primarily a cytoprotective mechanism, a massive autophagic response can cause cell death, also known as type II programmed cell death. Sphingosine kinase is an oncogenic enzyme that catalyzes the formation of pro-survival sphingosine-1-phosphate at the expense of pro-death ceramide, and is upregulated in a number of cancers, including acute myeloid leukemia (AML). SKI-1 is a novel non-lipid, selective small molecule inhibitor of sphingosine kinase with anti-tumor activity; however, the mechanism of action of SKI-1 is not fully understood.

Objectives: To elucidate the role of autophagy in SKI-1 induced cell death.

Design/Method: Atg5 knock out and wild-type mouse embryonic fibroblasts (MEF) as well as parental and vincristine resistant (VCR) AML HL60 cells were treated with SKI-1. Autophagy was determined by LC3 modification using Western blot, while apoptosis was evaluated by annexin V staining using flow cytometry and by cleavage of caspase-3 and PARP using Western blot. Cell viability was assessed by measurement of ATP production. Chloroquine, a lysosomal inhibitor was used to suppress autophagy, while zVAD-fmk, a pan-caspase inhibitor, was used to block apoptosis.

Results: 1) SKI-1 simultaneously induces apoptosis and autophagy. LC3 modification, a marker for autophagosome formation, was increased by SKI-1 treatment in MEF
wild-type as well as in HL.60 and HL.60/VCR cells. 2) Suppression of autophagy enhances SKI-I induced apoptotic cell death. Chloroquine increased SKI-I induced apoptosis in HL.60 and HL.60/VCR cells, as measured by annexin V staining, but did not show a clear decrease in cell viability, as measured by ATP levels. Aig5 knock out MEFS cells, which are deficient in autophagy, demonstrated enhanced cleavage of caspase-3 compared to wild-type cells. 3) Caspase inhibitor zVAD-fmk suppressed SKI-I induced apoptosis but did not affect ATP reduction induced by SKI-I. Conclusion: Our results show that SKI-I not only induces apoptosis but also autophagy. It seems that autophagy protects cells from apoptosis in response to SKI-I treatment. However, SKI-I may be able to induce both caspase-dependent and - independent cell death. Thus, manipulating autophagic activity may influence the efficacy of SKI-I in treatment of AML.

(POSTER 205) 2009 PANDEMIC H1N1 INFLUENZA A IN PEDIATRIC ONCOLOGY AND HEMATOPOIETIC STEM CELL TRANSPLANT PATIENTS: A REVIEW OF A SINGLE CENTER EXPERIENCE

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Background: Pediatric oncology and hematopoietic stem cell transplant (HSCT) patients are at high risk for severe disease and complications associated with seasonal influenza infection. H1N1 influenza infection in this population has not been described.

Objectives: To describe the clinical features and outcomes of H1N1 influenza A infection in pediatric oncology and HSCT patients at Children’s Medical Center Dallas.

Design/Method: Prospective laboratory surveillance identified all children with positive influenza testing from 4/27-11/15/2009. H1N1 infections were considered proven if H1N1 was confirmed by PCR subtyping; probable if no subtyping was performed. Retrospective review of medical records was performed for all pediatric oncology and HSCT patients. Non-parametric statistics were applied.

Results: Thirty of 615 (5%) patients (median age 10 years) with cancer or HSCT had influenza A infection. Twelve patients (4; 13%), or RVP (4; 13%) assays. Most common presenting symptoms were fever (38; 100%), cough (29; 97%) and rhinorrhea (25; 83%). Ten patients required hospitalization for 5 days for febrile neutropenia (8%; 80%), pneumonia (10%), or respiratory failure (10%). Inpatients had greater neutropenia (p = 0.03) and lymphopenia (p = 0.02) than outpatients. Chest x-rays demonstrated lower respiratory tract infection in 5 of 15 (20%) evaluable patients. There were no bacterial co-infections; 1 patient had rhinovirus co-infection. Four (13%) hospitalized patients experienced significant complications: 3 patients required ICU care for blood pressure support (2) and hypoxia (1). One developed ARDS and multi-organ failure requiring mechanical ventilation, ECMO, CVVH, and subsequently died. Chemotherapy was delayed in 5 hospitalized patients during their influenza illness. Oseletamivir was started in 28 patients. One patient who had received oseletamivir chemoprophylaxis developed an oseletamivir-resistant strain and was treated with zanamivir.

Conclusion: 2009 pandemic H1N1 influenza A infection occasionally can lead to complicated disease in children with cancer and HSCT, but is generally mild in most patients.

(POSTER 206) EVALUATION OF LATE CARDIOTOXICITY IN CHILDREN WHO RECEIVED LOW DOSE ANTHRACYCLINES FOR TREATMENT OF PEDIATRIC CANCER

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Background: Although pediatric cancer cure rates continue to improve, survivors who are exposed to anthracyclines during treatment may experience cardiac toxicity. It is well known that higher doses of anthracyclines can cause cardiotoxicity years after treatment. Low dose (LD) anthracyclines have been assumed to not be cardiotoxic, although the few studies that include patients who have received <100 mg/m2 provide conflicting results. The difficulty in interpreting these varied studies stems from the small patient numbers, the variable age at exposure (young age increases risk), and the range of follow-up after exposure as a short follow-up interval may fail
to detect cardiotoxicity. It is imperative to determine if there is cardiotoxicity after LD anthracyclines to allow proper education about long-term side effects for this group.

Objectives: To determine if children who received LD anthracyclines have cardiotoxicity by evaluating the fractional shortening (SF), left ventricular end diastolic dimension (LVEDd), and left ventricular posterior wall thickness (LVPWT).

Design/Method: A cohort of 52 cancer survivors who were under five years-old at diagnosis, at least six years from diagnosis, and received < 100 mg/m2 of anthracyclines underwent echocardiogram. We excluded children with CHD and history of radiation. LVPWT and LVEDd were converted into Z-scores based on BSA. One sample t-tests were used and < 0.015 was considered significant.

Results: The study included 26 females. The mean age at diagnosis was 2.66 years and mean time from diagnosis to echocardiogram was 9.63 years (6-16 years). The mean SF was 35.7% (NS). However, there were two patients with a SF < 28%. The LVPWT mean z-score was ~1.39 (p < 0.0001). The LVEDd was half a standard deviation from normal with a mean z-score of ~0.55.

Conclusion: Our study found that young children after LD anthracycline exposure had evidence of cardiotoxicity. They had a significantly decreased LVPWT, which is consistent with previous reports of increased afterload in young children exposed to anthracyclines. It is also concerning that two children had significantly decreased SF and six were less than normal. These findings are evidence that there is no safe anthracycline dose and that these children need long-term cardiac follow-up.

(POSTER 207) IMPROVING LEUKEMIA CARE DOCUMENTATION WITH THE ELECTRONIC MEDICAL RECORD

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Background: A typical boy with standard risk leukemia will require 19 intrathelial therapies, 1200 oral and 60 IV chemotherapy doses, 72 clinic visits, and 3 admissions. Treatment is delivered in the inpatient, outpatient, infusion clinic, and home health care settings. The electronic medical record (EMR) holds promise for efficient, standardized and flexible care for the subspecialty patient. We describe our use of a consolidated EMR to improve documentation compliance for leukemia patients.

Objectives: To utilize an EMR to improve oral chemotherapy documentation in the inpatient and outpatient settings.

Design/Method: Our method was to utilize Epic’s Beacon oncology module to implement a comprehensive chemotherapy ordering and documentation system. The EMR includes notes, laboratory, radiology, supportive care and chemotherapy orders. Generally, our implementation phases were plan, build, validate, train and optimize. Pre-EMR workflow included manual tracking, ordering, and documentation. Utilizing the Beacon EMR, a list of all patients with active chemotherapy is generated with independent weekly review and editing by the nurses, physicians, and pharmacists. Beacon orders populate EMR nursing flowsheets for medication reconciliation and documentation of missed doses based on the Beacon order. The physician reviews and confirms the plans.

Results: Twenty-eight Beacon protocols were created for the 4 open COG frontline ALL studies. Over 242 months of oral chemotherapy was administered during the study period (1/2009–12/2009). The 33 ALL patients receiving chemotherapy 1/1/2009 were transitioned to Beacon protocols, and all subsequent patients were treated utilizing the EMR. Five of eight new patients were eligible for experimental protocols and enrolled. Pre-EMR: Internal audit revealed deficient home oral medication documentation in 7 of 20 patients, and COG audit revealed deficient home oral medication documentation in 3 of 8 patients. Post-EMR: External independent and COG audit of ALL patients found no deficiencies or errors (no findings in the entire COG audit).

Conclusion: A small institution reports successful implementation of a comprehensive pediatric oncology ordering system. Through the use of the EMR we have improved medication documentation, maintained good patient care, and been favorably evaluated by outside auditors.

(POSTER 208) RISK FACTORS FOR CENTRAL LINE-ASSOCIATED BLOODSTREAM INFECTION IN PEDIATRIC ONCOLOGY PATIENTS

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Background: Central line-associated bloodstream infections (CLABSI) are a frequent complication of the use of central venous catheters (CVC) among pediatric patients with cancer but risk factors for CLABSI in this patient population remain poorly defined.

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OBJECTIVES: We sought to identify risk factors for CLABSI that might guide the application of adjunctive measures such as antibiotic-impregnated catheters or antiseptic-eluting dressings.

Design/Method: We retrospectively analyzed all identifiable cases of malaria in a population of 2010 ASPHO ABSTRACTS pediatric oncology patients with hematologic malignancies, a CVC with multiple lumens, or who are expected to require frequent platelet transfusions may benefit from adjunctive measures to decrease the risk of CLABSI.

Conclusion: Pediatric oncology patients with hematologic malignancies, a CVC with multiple lumens, or who are expected to require frequent platelet transfusions may benefit from adjunctive measures to decrease the risk of CLABSI.

(POSTER 209) MALARIAL EPISODES IN PAEDIATRIC ONCOLOGY PATIENTS IN SENEGAL: A RETROSPECTIVE, SINGLE-CENTRE STUDY

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Background: Myelosuppression is common amongst paediatric oncology patients but published literature is lacking on the effects this has on severity of malarial infection in these patients, from endemic areas.

Objectives: We hypothesized that myelosuppression would result in increased severity of malarial episodes in such patients. If confirmed, malarial chemotherapy could be adjusted to reduce malarial mortality in children receiving chemotherapy for acute leukemias.

Design/Method: We retrospectively analyzed all identifiable cases of malaria in a 10-year period (2000 to 2009) from a single institution (Le Dantec Hospital, Dakar, Senegal). Anonymized demographic and clinico-pathological data were recorded. Severe anaemia was defined as Hb < 6 g/dl; leucopenia as total WCC < 4 x 10^9/l; neutropenia as < 1 x 10^9/l and lymphopenia as non-neutrophil component < 2.5 x 10^9/l. Leukaemia patients at diagnosis were classified as functionally cytophenic for white cell components. Primary outcome was defined as patient recovery or death (where malaria was a contributory factor) within one month of diagnosis. Data analyzed in SPSS (v16.0) and by Fishers exact test.

Results: 55 malarial cases confirmed in 54 patients (from ~400 patients overall), whose diagnosis was lymphoma (20 cases; including 7 Burkitts, 5 Hodgkins), Wilms', leukemia (10 cases: 7 ALL, 3 AML), other malignancy (6). At one month, 50 (90.9%) patients had recovered and only 5 (9.1%) had died. Where counts available, 10/40 (25%) patients were severely anaemic (of whom 1 died), 15/40 (37.5%) leucopenic (0 died), 10/28 (35.7%) neutropenic (0 died) and 14/28 (50%) lymphopenic (0 died). No difference was detected by primary outcome measure for leucopenic (0 died), 10/28 (35.7%) neutropenic (0 died) and 14/28 (50%) patients were severely anaemic (of whom 1 died), 15/40 (37.5%) leucopenic (0 died), 10/28 (35.7%) neutropenic (0 died) and 14/28 (50%) lymphopenic (0 died). No difference was detected by primary outcome measure for leucopenic (0 died), 10/28 (35.7%) neutropenic (0 died) and 14/28 (50%) lymphopenic (0 died).

Conclusion: Despite the high incidence of myelosuppression in paediatric oncology patients, we found no evidence that this was associated with increased severity of malarial episodes. This study does not support the routine use of malaria chemotherapy in this population.

(POSTER 201) PERIPHERAL T CELL LYMPHOMA IN CHILDREN: A SINGLE CENTER EXPERIENCE IN COLOMBIA

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Background: Peripheral T Cell Lymphoma (PTCL) is a rare type of Non Hodgkin Lymphoma (NHL) classified as Anaplastic or Non-Anaplastic. PTCL can present as localized or disseminated disease, compromising skin, bones, lungs, liver, spleen, lymph nodes and bone marrow. PTCL occurs at any age in childhood, few reviews have focused on PTCL in young patients.

Objectives: The aim of this study was to describe the characteristics of PTCL at our institution.

Design/Method: A retrospective analysis was carried out of patients diagnosed with PTCL over a 14-year period (1995 to 2008). Demographics, clinical presentation and outcomes are described. Diagnoses were based on morphology and immunophenotype with CD2, CD3, CD4, CD5, CD7, CD8, CD20, CD25 and CD30 markers.

Results: The series included 26 patients, which corresponded to 17% of all patients < 18 years of age diagnosed with NHL in our unit (n = 153). Fourteen (54%) were male and 12 (46%) were female with a median age at diagnosis of 9.4 years (range: 3–16).

The most common sites compromised were: lymph nodes n = 17 (65.4%), spleen and liver n = 6 (23%), skin n = 4 (15.4%), lung n = 3 (11.5%), nasal sinuses n = 3 (11.5%), bone n = 1 (3.8%) and bone marrow n = 1 (3.8%).

Fifteen cases were classified as Anaplastic (57.6%) and 11 as Non-Anaplastic (42.4%). Histological subtypes in the latter group were: non-specific in 4 (15.2%), extranodal nasal NK/T in 3 (11.5%), subcutaneous panniculitis-like in 2 (7.7%), angiocentric in 1 (3.8%) and angioimmunoblastic in 1 (3.8%).

Twelve patients were in stages II-IV (46%) and 14 patients were in stages III-IV (54%).

Patients were treated with multiagent chemotherapy (CHOP, MACOP-B and NIH-BFM).

Thirteen patients (50%) were alive at the time of last follow-up in 2008, 12 were dead (46%) and 2 lost of follow-up(4%). Deaths occurred in 72.3% of Non-Anaplastic, in 33.3% of Anaplastic and in 66.6% of cases with advanced disease.

Conclusion: PTCL is uncommon, frequently having an aggressive behavior. The response to conventional chemotherapy regimens in Non-Anaplastic-PTCL is poor. The best approach for advanced PTLC has not been clearly defined. International collaboration and further understanding of the biology of these lymphomas is necessary to improve overall survival.

(POSTER 211) THE ASSOCIATION OF HOSPITAL CONSTRUCTION WITH THE DEVELOPMENT OF ENVIRONMENTAL HEALTH-ASSOCIATED MOLD INFECTIONS (EHCAMI) IN CHILDREN WITH ACUTE LEUKEMIA

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Background: Invasive fungal infections (IFI) cause significant morbidity and mortality in children receiving chemotherapy for acute leukemias (AL). From April 2006 until February 2007, Children’s Medical Center of Dallas (CMCD) underwent excavation adjacent to existing buildings. Precations to minimize exposure to aerosolized spores included re-sealing windows before excavation, valet parking for oncology patients, use of N-95 respirators outside HEPA-filtered areas, and daily watering of dirt at excavation sites. Audits of adherence to the use of valet parking and N95 respirators were not performed.

Objectives: We analyzed the association of excavation on environmental health care associated mold infections (EHCAMI) in comparison with its association with yeast infections in the same population during the same time period.

Design/Method: We conducted a retrospective review of all patients (N = 275) receiving intensive chemotherapy for AL at CMCD from 2004-2008. Exposure was defined as 1 clinic visit or 1 in-patient day; the presence of neutropenia was documented for each exposure. All IFI were categorized using EORTC/MSG guidelines for proven (n = 34), probable (n = 0) and possible infections (n = 16). Infection control definitions classified EHCAMI as definite (n = 8), probable (n = 0) and possible (n = 23). Multiple-events time-to-event models and repeated-measures logistic regression models compared the association of excavation with EHCAMI and yeast infections, controlling for neutropenia.

Results: There were 7454 CMC exposures, 1007 during excavation. Fifty IFI were identified, 31 were EHCAMI. Time-to-event analysis, controlling for neutropenia, found that excavation was significantly associated with EHCAMI (HR = 2.8, P = 0.01) but not with yeast (HR = 0.75, P = 0.75). The analysis, however, did not rule out that these associations were equal (P = 0.24). Each day of neutropenia was significantly associated with the development of both yeast and EHCAMI (HR = 1.1, P < 0.01). Similar results were found by logistic regression: excavation was marginally associated with EHCAMI (P = 0.07) and not with yeast (P = 0.65), while neutropenia was significantly associated with both (P < 0.001).
Conclusion: Our patients, exposed to excavation, continued to develop EHCAMI. Strict monitoring of adherence to preventive strategies is needed to determine their effectiveness and if additional strategies are required.

(PAPER 212) GROWTH HORMONE DEFICIENCY AFFECTS A SIGNIFICANT POPULATION OF T-ALL SURVIVORS

Linda Butros, Stuart Winter, Susan Scott MD
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Background: T lineage acute lymphoblastic leukemia (T-ALL) requires a distinct treatment approach in order to be successful. Specifically, relapse into the CNS is a common problem that is often addressed with the addition of whole brain radiation to the treatment regimen.

Objectives: We hypothesized that survivors of T-ALL might have a significant risk for growth hormone (GH) deficiency due to the routine addition of cranial radiation for CNS prophylaxis.

Design/Method: We reviewed our database of 146 survivors of childhood leukemia and identified 17 patients who were treated for T-ALL on or as per COG 9404, 123, CCSG 132, and POG 9412. The database includes patients who are at least two years off treatment and free of disease.

Results: Of these seventeen T-ALL survivors, five patients (29%) had overt signs of pituitary GH deficiency. Two more survivors had low IGF-1, but further characterization for GH deficiency was not able to be accomplished due to lack of compliance. The treatment characteristics and laboratory values for the five T-ALL survivors with growth hormone deficiency are listed in Table 1 below. The normal ranges for IGF-1 and IGF-BP3 are listed in parentheses.

Conclusion: These results suggest that GH deficiency is common in patients treated for T-ALL. GH deficiency appears to be over-represented in survivors of T-ALL as compared to pre-B ALL although further analysis is needed. We speculate that the interaction between higher-dose methotrexate and whole brain radiation may predispose to this particular long term side effect of treatment in survivors of T-ALL. T-ALL survivors require careful monitoring for GH deficiency through biannual measurements of serum IGF-1 and IGF-BP3 as well as plotting of height, weight, and body mass index for age at annual physical examinations. Future studies are needed to further elucidate the risks for GH deficiency in T-ALL survivors.

Tables/Charts:

<table>
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<tr>
<th>Patient</th>
<th>CNS radiation</th>
<th>Treatment regimen</th>
<th>Bone marrow transplant</th>
<th>Age at diagnosis</th>
<th>Months off treatment</th>
<th>IGF-1</th>
<th>IGF-BP3</th>
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*Multiple endocrine deficiencies after transplant including sex hormone and thyroid hormone deficiencies.
** Failed growth hormone stimulation test.

(PAPER 213) EFFECTIVE USE OF SINGLE DOSE OF DARBEPOETIN ALPHA IN REDUCING BLOOD TRANSFUSION DURING INDUCTION CHEMOTHERAPY IN LEUKEMIC CHILDREN

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Background: Anemia is usually seen in children with malignancy. Etiology is multifactorial. It may be from the disease itself, the treatment or from infection. Even though children tolerate anemia better than adults however, their anemia is usually aggravated by the use of intensive Chemotherapy and they often require multiple blood transfusion during their induction/consolidation phases. Recombinant human erythropoietin is used effectively in anemia in chronic renal failure and in adult cancer patients. The data on Darbepoetin in leukemic children are lacking.

Objectives: To determine the effectiveness and safety of a single dose of Darbepoetin alpha in reducing the need of packed cell transfusion during the induction chemotherapy phases in children with Leukemia.

Design/Method: Study was conducted in Sultan Qaboos University Hospital pediatric hematology ward from July 2007 to November 2009. These children were compared retrospectively with an earlier group of 25 children who did not receive Darbepoetin alpha with matched age, sex and diagnosis (control group). The children were evaluated on daily basis for possible side effects especially high blood pressure and thrombosis.

Results: Total number of children in the study were 26. All were diagnosed with different types of acute lymphoblastic Leukemia. Males were 12 while females were 14. Their Age ranged between 2yrs to 12 years. All of them admitted with Hb less than 9g/dl and were transfused once before the diagnosis. All were given Darbepoetin alpha of 2.5ug/kg in the first week of induction. Out of these seven children (27%) didn’t need any transfusion while 14 children needed one blood transfusion (54%) during four week chemotherapy. Five (19%) children needed two transfusions. There were no side effects reported related to the use of darbepoetin. In the control children 5 (20%) received one transfusion and 20 (80%) of them needed more than two blood transfusions. Mean transfusion requirements during induction in the study group was 245 ml, and in the control group was 635ml (P < .001).

Conclusion: Use of single dose of Darbepoetin during four weeks induction was effective and safe in reducing blood transfusion during the intensive chemotherapy.

(PAPER 214) HISTIOCYTIC SARCOMA DURING MAINTENANCE CHEMOTHERAPY FOR PRECURSOR B ACUTE LYMPHOBlastic LEUKEMIA: CASE REPORT WITH EVIDENCE FOR A COMMON CLONAL ORIGIN

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Background: Histiocytic sarcoma (HS) is a rare neoplasm with a uniformly poor prognosis. Although secondary malignancies are relatively common in leukemia patients, few bona fide cases of HS have been reported.

Objectives: We present a 4 year old male who developed HS while receiving maintenance chemotherapy for pre-B ALL. Polymerase chain reaction (PCR) studies of the immunoglobulin heavy chain (IgH) and T-cell receptor-gamma (TCRg) gene loci showed identical patterns in the leukemic blasts and the HS cells.

Design/Method: Case report and review of literature.

Results: A 4 year old male was referred to the Mayo Clinic, for a second opinion regarding a recent diagnosis of HS. The patient had been diagnosed with standard risk, pre-B ALL in September of 2007 and was enrolled on the COG study AALL0331. Shortly after starting maintenance chemotherapy, he developed diffuse, bilateral leg pain. A plain film demonstrated a 2.7 x 2.2 x 1.7 cm lytic lesion in the right distal femoral metaphysis. Findings were confirmed on MRI scan and patient underwent an open biopsy of the lesion. Morphologic evaluation demonstrated large, anaplastic cells with abundant eosinophilic cytoplasm. By immunohistochemical staining, the tumor cells were positive for CD68, CD163, CD4, and CD43RO, with weak focal positivity for S-100 and myeloperoxidase. A diagnosis of HS was made. PCR studies of the IgH gene regions (fr3, fr3a, fr3b) each showed strong clonal bands in both the HS and the previous B-ALL. PCR for the TCRg gene also demonstrated identical bands both in the HS cells and the patient’s previous ALL, indicating a clonal relationship between the two neoplasms. Leukemia therapy was held and patient was started on chemotherapy as per AIEOP-ALCL 1999. Patient developed widespread metastasis on chemotherapy and was placed on palliative care.

Conclusion: The molecular studies in this case indicate a common clonal origin of the patient’s ALL and HS. While the exact mechanism of lineage switch from a lymphoid neoplasm to a histiocytic one remains unclear, mouse studies have indicated that altering expression of lineage-associated transcription factors such as PAX5 may cause the “re-programming” of lymphoid cells to either macrophages or uncommitted progenitor cells.

(PAPER 215) ADDRESSING THE BARRIERS OF NEUROCOGNITIVE SCREENING IN LONG-TERM SURVIVORS OF PEDIATRIC CANCER: A BRIEF NEUROCOGNITIVE SCREENING METHOD

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Background: Advances in pediatric oncology have resulted in significant improvement in disease-free survival with 80% of children with cancer expected to be long-term survivors. Studies have shown that a large percentage of childhood cancer survivors are at risk for neurocognitive impairment. Currently Children’s Oncology Group (COG) recommends screening of all patients at risk for neurocognitive deficits upon entry to a post-chemotherapy clinic. Obstacles to addressing this goal include lack of institutional resources and extensive wait time to be seen by a neuropsychologist.
**OBJECTIVES:** To retrospectively review a brief neurocognitive screening method as a means to evaluate and refer high risk survivors of childhood cancer for appropriate neurocognitive interventions.

**Design/Method:** Upon entry to the Keyser Family Center Long-Term Follow-Up Clinic, trained social workers, teachers and nurses administer a brief neurocognitive screening evaluation lasting approximately 20-30 minutes. The assessment combines the following neuropsychological testing methods: Grooved Pegboard, Wechsler Intelligence Scale for Children: Digit Span, Wide Range Assessment of Memory and Learning, Second Edition (WRAML-2); Story Memory, Controlled Oral Word Association Test (COWA) and the Trail Making Test. Testing identifies functional deficits including executive function, sustained attention, memory, processing speed and visual-motor integration, allowing us to make appropriate referrals. Test results and scores are subsequently interpreted by a trained neuropsychologist followed by further recommendations if needed.

**Results:** Within our population of approximately 177 childhood cancer survivors, we performed this unique screening on 44% (78) of the patients. 73% of patients screened had the diagnosis of leukemia or lymphoma, with 93% having received intrathecal chemotherapy. Of those patients screened, we referred 49% to the school system for successful implementation of necessary interventions (i.e. IEP and 504 plans). 5.6% required referral for full neuropsychological evaluation; several, with concomitant emotional issues.

**Conclusion:** Performing this brief evaluation, we were able to successfully identify patients suffering from neurocognitive sequelae and offer appropriate referrals to both the school system and neuropsychologist. Our data demonstrates the value of this method in screening survivors of pediatric cancer for neurocognitive deficits, as well as successfully addresses some of the barriers in achieving appropriate neurocognitive care.

**POSTER 216**

**PREVALENCE OF FLT3 INTERNAL TANDEM DUPLICATION IN PEDIATRIC PATIENTS WITH ACUTE MYELOID LEUKEMIA AT CHILDREN CANCER HOSPITAL EGYPT (CCHE)**

Nayera Hamdy, Sherine Salem, Dina Yassin, Sonia Mahmoud, Lobna Shalahy, Manal Zamzam, Hany Hussein, Hany Abdul Rahman, Emad Moussa, Iman Attia, Madeha Mahmoud, Enas El-Nadi, Alaa Elhaddad, Mohamed Sedky, Khaled Shaaban, Tarek Mansour, Sherif Abeolnaga, Sonya Soliman, Hala Reda, Sahar Khaleel

CCHE 57357, Cairo, Egypt

**Background:** FLT3 is a class III receptor tyrosine kinase. FLT3 mutations were first reported as internal tandem duplication (FLT3/ITD) of the juxtamembrane domain-coding sequence, it occurs in 15% to 35% of patients with AML. FLT3/ITD is an important marker in risk stratification & treatment strategies of AML patients.

**Objectives:** To evaluate the prevalence of FLT3 mutation-internal tandem duplication in acute myeloid leukemia (AML) patients treated at CCHE & to compare FLT3/ITD positive cases to negatives ones regarding demographics, FAB subtype & other known cytogenetic abnormalities.

**Design/Method:** We have screened 152 pediatric patients with newly diagnosed AML; who have presented to Children Cancer Hospital Egypt (CCHE) during the period from July 2007 to July 2009; for the presence of FLT3/ITD by PCR. For cases positive for the mutation, the allelic ratio of FLT3/ITD (ITD-AR, mutant-wild type ratio) was determined using densitometric analysis.

**Results:** FLT3/ITD was detected in 11.8% (18 patients). The FLT3/ITD-AR of the positive cases ranged from 0.43 to 2.26, with a median of 0.92, with 8 patients having FLT3/ITD-AR greater than 1. The mutation was equally distributed among males and females, and the median age of patients was 10 years. FLT3/ITD was not equally distributed among various FAB subtypes. Its frequency was ranked as: M2 (44%) > M3 (22%) & M4 (22%) > M1 (11%). None of M5, M6 or M7 FAB subtypes had the mutation. The total leukocytic count for patients positive for the mutation ranged between 3.2 – 444 × 10^9/L, with a median of 78.15 < 10^9/L. Among FLT3/ITD positive patients, 33% had favorable risk stratification (2 cases 0/21), 4 cases (15/17), 6% had high risk stratification (1 patient 0/6/9), and the rest had intermediate risk stratification (61%).

**Conclusion:** This study shows that FLT3/ITD is common among Egyptian pediatric AML patients and represents biologically heterogeneous subtypes of the disease.

**POSTER 217**

**IMPROVING PEDIATRIC SURVIVORSHIP CARE: A NEEDS-BASED ASSESSMENT OF GENERAL PRACTITIONERS REGARDING LATE EFFECTS OF CHILDHOOD CANCER TREATMENT IN ADULT SURVIVORS OF PEDIATRIC CANCER**

Kelly McCrann, Kerry Moss, Linda Overbolser, Jean Kutner, Brian Greffe, Lila Gore

The Children’s Hospital/University of Colorado, Aurora, Colorado, United States

**Background:** Improvement in the rate of care for childhood cancers has significantly increased the number of adult survivors of childhood cancer living in the United States. Unfortunately, many of these patients do not receive recommended survivor based follow-up and furthermore do not have established primary care. Issues surrounding the effective transition of care from pediatric oncologists toward general practitioners are of paramount importance.

**Objectives:** This study aimed to investigate the experience, knowledge and interest of general practitioners in regards to providing care for adult survivors of childhood cancer. The secondary aim of this study was to provide an understanding of the barriers preventing a more effective transition of care within this patient population.

**Design/Method:** An online survey composed of twenty-five questions was emailed to approximately 1,800 internists and family practitioners in Colorado assessing their training, knowledge and interest regarding the late effects of childhood cancer therapy and further investigating their comfort in providing care for this patient population.

**Results:** Forty-seven physicians completed the survey. The majority (78.7%) indicated no training in the area of late effects and ever ninety percent responded that further education would improve their care of this patient population. When presented with knowledge-based questions regarding childhood cancer survivorship and late effects, responses varied greatly and physicians replied “I don’t know” nearly one-third of the time (29.0%). Furthermore, low levels of comfort were reported among the physicians, with nearly half (48.9%) stating discomfort with recognizing and diagnosing late effects.

**Conclusion:** Despite expressing interest in this area of medicine, general practitioners lack education regarding the late effects of childhood cancer treatment and have minimal comfort in treating this patient population. Improving the healthcare of adult survivors of childhood cancer relies upon an effective transition of care as well as the education of the providers assuming their care. While current practice places emphasis on the empowerment of survivors to both understand their previous treatment and seek appropriate healthcare, this study demonstrates the additional importance of improving the awareness and education of the general practitioners providing their care.

**POSTER 218**

**PERIORAL AND FACIAL PARESTHESIAS ASSOCIATED WITH INTRAVENOUS PENTAMIDINE USE FOR PNEUMOCYSTIS PROPHYLAXIS**

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Medical University of South Carolina, Charleston, South Carolina, United States

**Background:** Pentamidine is efficacious for second line chemoprophylaxis of Pneumocystis jiroveci (PCP) pneumonia in the immunocompromised host. Pentamidine associated neurologic side effects are extremely rare and have not previously been reported in pediatrics. Postulated mechanisms include a direct neuropathic effect of the drug or a manifestation of a transient metabolic abnormality such as hypocalcemia and hypoglycemia produced by the drug.

**Objectives:** To report five cases of perioral and facial paresthesias associated with pentamidine.

**Design/Method:** Case series

**Results:** From 7/2008 through 10/2009, 19 patients at MUSC have received at least one pentamidine dose with five patients experiencing paresthesias. Documented adverse events occurred with 10 of 133 total doses administered for an adverse event rate of 7.5 % (10/133 doses). All patients received pentamidine (4 mg/kg) IV over an hour. Symptoms including facial numbness, perioral numbness, and extremity paresthesias occurred at the end of the infusion or immediately post-infusion while one patient developed perioral numbness and left hand paresthesias 15 minutes into the infusion. These episodes resolved within 30 minutes of completion or interruption of the infusion with each patient able to complete their infusion. Three patients continue to receive monthly IV pentamidine with one experiencing facial paresthesias with each subsequent dose, another with no repeat paresthesias after initial dose, and one with serial paresthesias with initial and subsequent infusion. One patient accumulated to disease prior to repeat infusion and another was switched to aerosolized pentamidine without repeat occurrences. Four patients had serum electrolytes obtained before and after their initial episodes. Only one patient had an electrolyte disturbance; mild transient hypocalcemia (serum calcium of 7.4 mg/dL, from 8.9mg/dL). The first three observed cases occurred during a one week time span and these patients received pentamidine from the same lot number. How ever, the lot was discarded and the other two cases occurred 9 months and 14 months after the initial case with a new lot of pentamidine.

**Conclusion:** The mechanism for pentamidine induced paresthesias remains unclear. Pediatric Oncologists should be aware of these potential adverse events in children receiving pentamidine. Fortunately, these pentamidine-associated paresthesias do not appear to be serious and are self-limiting.
Background: Mastocytosis encompasses a spectrum of disease characterized by abnormal proliferation of mast cells (MC). Cutaneous Mastocytosis is seen in young children and is typically benign. Systemic mastocytosis (SM) is a persistent, multi-organ-system disorder seen almost exclusively in adults. Associated hematologic, non-MC lineage, disease (AHNMD) occurs concurrently in up to 20% of SM cases. AHNMD can include acute myeloid leukemia (AML), which demonstrates (t;8;21). In SM-AHNMD, the patient must meet criteria for both SM and AHNMD.

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Myelomastocytic leukemia includes patients with advanced myeloid neoplasms who demonstrate an increase in immature atypical mast cells, but do not meet the criteria for SM or for mast cell leukemia. The youngest reported patient with mastocytic disease and AHNMD was 17 years old.

Objectives: A 9 year old male was referred to the pediatric hematology/oncology division at Mayo Clinic with a mediastinal mass. Bone marrow biopsy revealed a population of T lymphoblasts (TdT, CD3, CD34, CD117, CD10, CD2, CD4, CD5 positive; MPO negative) and a separate distinct population of myeloid blasts (MPO, CD33, CD4 positive; TdT, CD3, CD34, CD4 negative). In addition to a 9q deletion, a previously unreported mutation, (t;5;14)(q35;32) leading to HOX11L2/BCL11B fusion was observed. FISH studies showed the anomaly in both populations. Extrapolating data from hiperphenotypic leukemia, we elected to treat the patient with ALL based chemotherapy. He achieved complete remission with induction chemotherapy, and subsequently received one course of consolidation chemotherapy followed by an unrelated cord blood transplant. Pre-transplant conditioning consisting of fludarabine, cyclophosphamide and TBI. Cyclosporine and mycophenolate were administered for GVHD prophylaxis. Patient engrafted successfully and day 100 marrow revealed complete donor chimism. Patient is currently 220 days post transplant and continues to remain in remission.

Conclusion: Majority of leukemias can be assigned to myeloid, B- or T- lineage. aBLL represents a very small subset where 2 distinct blast populations can be identified. Data on management is anecdotal, and the prognosis uniformly dismal. It is unclear whether these patients should be treated with ALL or AML based chemotherapy, or both. This case documents the detection of (t;5;14)(q35;q32) HOX11L2/BCL11B for the first time in aBLL with myeloid and T-cell differentiation. Also, our patient achieved complete remission with ALL based induction chemotherapy and remains in remission following an unrelated cord blood transplant.

**RESULTS:** The patient did not meet all criteria for SM-AHNMD and closely fit the classification of SM with aHMD (t;5;14)(q35;q32). Our patient was successfully managed with ALL chemotherapy and achieved complete remission.

**CONCLUSION:** Acute Bilineal Leukemia (aBLL) is characterized by the simultaneous presence of 2 distinct populations of blasts each expressing a lineage specific pattern of differentiation. It accounts for less than 1% of acute leukemias and has been previously associated with 9;22 and 11q23 abnormalities. Prognosis, despite multiagent chemotherapy and stem cell transplantation is poor with only 2 long term survivors reported in literature.

**OBJECTIVES:** We report a pediatric case of aBLL with a previously unreported novel mutation. Our patient was successfully managed with ALL based chemotherapy and cord blood transplant.

**Design/Method:** Case report.

**RESULTS:** A 9 year old male was referred to the pediatric hematology/oncology division at Mayo Clinic with a mediastinal mass. Bone marrow biopsy revealed a population of T lymphoblasts (TdT, CD3, CD34, CD117, CD10, CD2, CD4, CD5 positive; MPO negative) and a separate distinct population of myeloid blasts (MPO, CD33, CD4 positive; TdT, CD3, CD34, CD4 negative). In addition to a 9q deletion, a previously unreported mutation, (t;5;14)(q35;32) leading to HOX11L2/BCL11B fusion was observed. FISH studies showed the anomaly in both populations. Extrapolating data from hiperphenotypic leukemia, we elected to treat the patient with ALL based chemotherapy. He achieved complete remission with induction chemotherapy, and subsequently received one course of consolidation chemotherapy followed by an unrelated cord blood transplant. Pre-transplant conditioning consisting of fludarabine, cyclophosphamide and TBI. Cyclosporine and mycophenolate were administered for GVHD prophylaxis. Patient engrafted successfully and day 100 marrow revealed complete donor chimism. Patient is currently 220 days post transplant and continues to remain in remission.

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**Background:** Intravascular devices (IVD) are commonly used in the care of pediatric patients with a hematologic or oncologic diagnosis. Catheter-related bloodstream infections (CRBSI) are a known complication of IVD and frequently lead to removal of the device despite adequate antibiotic administration. In patients with limited vascular access, ethanol lock therapy (ELT) along with systemic antibiotics can offer the advantage of salvaging infected IVDs.

**Objectives:** Evaluate the efficiency of short-dwell ELT (70% Ethanol for 4 to 25 hours dwell time) in combination with systemic antibiotics for the treatment of CRBSI in hematologic/oncology patients.

**Design/Method:** A retrospective chart review was performed on patients up to 19 years of age with a hematologic or oncologic diagnosis in a prospectively collected CRBSI cohort. Patients who underwent ELT at Children’s Hospital of Michigan from 1/1/2007-7/15/2009. Primary outcome was sterilization of the infected IVD after ELT, defined by a negative blood culture.
culture from the infected IVD < 25 hours after ELT. Secondary outcomes measured were: recurrence of CRBSI within 30 days after ELT, successful retention of IVD for culture from the infected IVD < 828.

**Results:** Subjects were 39 patients ages 9m – 19 years (mean S.D. 8.9±6.3 years; 26 males) with 50 episodes of CRBSI. Underlying diagnoses were: Acute myelogenous leukemia (7), osteosarcoma (5), acute lymphoblastic leukemia (4), hemophilia (4), Hodgkin’s lymphoma (4), medulloblastoma (3), retinoblastoma (2), sickle cell disease (2) and others (8). Overall, 23% were status post bone marrow transplant. 82% of patients were immunosuppressed and 32% were neutropenic. The most commonly isolated pathogen was Staphylococcus epidemidum (36%); 6% were documented fungal CRBSIs and 24% were polymicrobial. ELT with systemic antibiotics successfully cleared 88% of CRBSIs. Mean duration of antimicrobials was 11.4days±4.5d. 82% of cases of CRBSIs had no recurrence within 30 days. IVD retention rate was 84%. ELT was well tolerated with no laboratory evidence of liver dysfunction.

**Conclusion:** Short-dwell ELT in combination with systemic antibiotic therapy appear to be an effective method of treating CRBSI and salvaging infected IVDs even in immunocompromised pediatric patients with a hematologic or oncologic diagnosis. Larger randomized studies are needed.

**POSTER 223**

**SURVEILLANCE FOLLOWING HEAD, NECK AND CHEST RADIOTHERAPY: THYROID ULTRASOUNDS MONITORING FOR SECONDARY THYROID MALIGNANCY**

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**Background:** Children who receive radiotherapy of the head, neck or chest as treatment for various types of primary malignancies are at increased risk for secondary cancers, especially thyroid gland malignancy. Thyroid nodules can be difficult to identify by physical exam and/or lab tests; however, thyroid ultrasound can be a non-invasive tool to detect new or growing nodules that are not palpable by exam.

**Objectives:** To evaluate the usefulness of thyroid ultrasounds in the surveillance of pediatric cancer survivors who have received head, neck or chest radiotherapy.

**Design/Method:** At Hope Children’s Hospital, we performed a retrospective chart review of pediatric cancer survivors to determine the usefulness of thyroid ultrasound to detect potential thyroid malignancy in patients who had received head, neck or chest radiotherapy.

**Results:** Children in our study received radiation between the ages of 8 months and 18 years, and had a variety of primary cancers with subsequently varying amounts of radiotherapy. The largest population of patients had Hodgkin’s lymphoma followed by those who received radiotherapy prior to bone marrow transplantation, and for a mixture of CNS tumors, other hematogenous cancers and solid tumors. The radiation dose ranged from 1200cGy to 5580 cGy. In our cohort of 47 patients who received radiotherapy, 36 (77%) had thyroid ultrasounds. Seventeen (47%) of those children had ≥1 nodule(s) detected on ultrasound. Seven (41%) of these patients went on for thyroidectomy (1 partial thyroidectomy), four of which were found to have thyroid malignancy. Specific pathology reports were reviewed for all patients. The remaining 10 patients continue to receive thyroid evaluation, including ultrasound, every 6–12 months, to monitor growth of the nodule(s).

**Conclusion:** This retrospective review suggests that routine use of thyroid ultrasound in high-risk patients is able to detect non-clinical thyroid nodules and potentially thyroid malignancy post-chemotherapy. As the number of childhood cancer survivors continues to increase, long-term follow-up guidelines have become imperative; thus, we propose that this non-invasive and non-harmful modality be incorporated into routine pediatric surveillance of all long-term survivors with history of head, neck or chest radiation.

**POSTER 224**

**SYSTEMIC MASTOCYTOSIS IN A CHILD WITH t(8;21) ACUTE MYELOID LEUKEMIA**

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**Background:** Systemic mastocytosis (SM) is characterized by the proliferation of mast cells in at least 1 extracutaneous organ. SM has been associated with t(8;21) Acute Myeloid Leukemia (AML) in adults but has not previously been reported in pediatrics.

**Objectives:** We report the case of a 10-year-old female with AML who developed asymptomatic SM following 3 cycles of chemotherapy.

**Design/Method:** This previously healthy female presented with abdominal pain and fever. Physical exam showed pallor and splenomegaly. Skin exam was clear. Her leukocyte count was 28.1 k/uL, hemoglobin was 9.3 g/dL, and platelets were 5 k/uL. A bone marrow biopsy (BM) revealed 77% myeloblasts. Immunophenotyping by flow cytometry revealed blasts which were CD34+, CD117+, CD3+, CD33+, HLA-DR+ and MPO+ and CD10–, TDT–, CDYTOPLASMIC MU–, CD22–, CD79A– and did not express surface T-Cell markers. Cyto genetic analysis revealed AML (8;21)(q22;q22). This was consistent with AML. She began treatment according to the Children’s Oncology Group AAML 0531 (standard arm). She did not achieve remission following two cycles of induction (intravenous cytarabine, daunorubicin and etoposide). Following intensification I (intravenous cytarabine and etoposide) her BM showed only 2% myeloblasts.

**Results:** Surveillance BM following intensification II (intravenous cytarabine and mitoxantrone) showed no residual leukemia but 21% abnormal myeloid cells which were CD33+, CD117+, CD45+, CD2- and CD34–, CD35– and MPO–. The immunophenotype was consistent with mastocytosis. She also had elevated serum tryptase at 20ng/mL (2-10ng/mL). No c-kit mutations were detected on exons 8,9,11, 13 and 17(B16) was negative. She received intensification III (intravenous cytartarabine and E. coli L-Asparaginase) and a matched-sibling allogeneic hematopoetic stem cell (HSCT) donor was identified. Her family opted against HSCT. After five months, she remains in remission of her AML with persistent SM stable at 20%.

**Conclusion:** SM may develop following treatment for AML. SM with t(8;21) AML leads to poorer outcomes among adults and HSCT has been recommended, especially for those with c-KIT mutations. While our patient did not have a c-KIT mutation, HSCT was recommended given her initially refractory AML and likely poorer prognostic status with associated SM. Prompt diagnosis, intervention and reporting of outcomes will be important in the establishment of treatment guidelines for affected pediatric patients.

**POSTER 225**

**CDS6 EXPRESSION AS A PROGNOSTIC INDICATOR IN PEDIATRIC AML: A SINGLE CENTRE REVIEW**

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**Background:** Modern treatment of pediatric acute myeloid leukemia (AML) involves intensive therapy modified according to predictive risk factors, including cytogenetics, molecular markers and treatment response. Multiple adult AML series have suggested blasts positive for neural cell adhesion molecule CD56 (CD56+) are associated with poor prognosis, but little is reported in pediatric AML.

**Objectives:** Our primary objective was to document the percentage of cases of de novo AML patients at our institution between 2002 and 2009 that were CD56+. Our secondary objective was to examine the relationship of CD56 expression with overall survival (OS) and event free survival (EFS).

**Design/Method:** We reviewed all cases of de novo AML in our institution from January 2002 to July 2009. Descriptive and summary statistics were calculated for our study population that was then divided into two groups based on CD56 status (positive versus negative). Survival was analyzed using the Kaplan-Meier method, with the Wilcoxon log rank test (LR) used for comparison between groups. The relationship of CD56 expression with OS and EFS was further examined using the Cox proportional hazards model for both univariate and multivariable analyses.

**Results:** We identified 73 cases of AML suitable for review, and 16 (22%) were CD56+. The 3-year OS was 75.0% (95%CI: 53.8, 96.2) and 82.5% (95%CI: 71.4, 94.0) for the CD56+ and CD56– group respectively (p = 0.15), and the 3-year EFS was 55.6% (95%CI: 31.4, 79.8) and 65.2% (95%CI: 51.8, 78.6) for the CD56+ and CD56– group respectively (p = 0.30). In univariate analysis, the relationship between CD56 status and survival was not statistically significant (OS: HR = 1.89, p = 0.30, EFS: HR = 1.45, p = 0.40). When adjusted for cytogenetic risk the relationship between CD56 status and survival remained non-significant (OS: HR = 1.83, p = 0.33, EFS: HR = 1.41, p = 0.44).

**Conclusion:** Twenty-two percent of children with de novo AML at our institution were CD56+ during our study period. CD56-positivity did not significantly affect OS or EFS on univariate and multivariable analysis. Our multivariable modeling was limited by our small sample size and unavailability of some potential confounders (such as response to treatment), therefore a larger, prospective study should be conducted in order to further examine the prognostic significance of CD56+ in pediatric AML.

**POSTER 226**

**MANAGEMENT OF POST-TRANSPLANT LYPHOPROLIFERATIVE DISORDER RESEMBLING PRIMARY CNS LYMPHOMA IN A PEDIATRIC RENAL TRANSPLANT PATIENT**
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Background: Post transplant lymphoproliferative disorder (PTLD) is a recognized complication of immunosuppression in solid organ transplant patients. A majority of cases are B cell in origin and associated with Epstein-Barr virus (EBV+). Presentation varies from benign lymphoid hyperplasia to aggressive fulminant lymphoma. Although any organ system can be involved, central nervous system (CNS) involvement is uncommon. PTLD isolated to the CNS is very rare and usually resembles monomorphic high-grade lymphomas. Only small cases series exist in the literature and currently there is no standardized therapy.

Objectives: We describe a case of EBV+ PTLD resembling diffuse large B cell lymphoma (DLBCL) isolated to the CNS and the rationale of our therapeutic approach.

Design/Method: Clinical, radiographic, histologic, immunophenotypic and molecular details of the case are provided. We compared our case to the clinical presentations, biology and outcomes of other reported cases of CNS PTLD.

Results: Thirty-three years following renal transplantation for congenital hydronephrosis, a 15 year-old male presented with seizures and right-sided weakness. MRI of the brain demonstrated multifocal ring enhancing hypointense lesions in the subcortical white matter associated with vasogenic edema. Biopsy showed monomorphic population of large lymphoid cells with irregular nuclear contours, minimal cytoplasm, and frequent mitotic figures resembling DLBCL. The tumor cells had a high proliferation index (~80%), and were positive for LCA, CD20, and occasionally CD79a, but negative for BCL-2, TdT, ALK-1. In situ hybridization for EBER1 was positive in the majority of cells. Immunosuppression was held for six weeks while chemotherapy was administered. Systemic chemotherapy included dexamethasone, methotrexate and rituximab. Intrathecal chemotherapy delivered via Omya reservoir included hydrocortisone, cytarabine, methotrexate and rituximab. Following two cycles of chemotherapy his neurologic examination stabilized and MRI demonstrated good partial response. Therapy was stopped after two cycles due to renal toxicity and immunosuppression was resumed at previous dosing. Serial MRI evaluations over the following nine months demonstrated resolution of CNS lesions with no further therapy.

Conclusion: Monomorphic PTLD isolated to the CNS is rare in pediatric and is associated with poor outcome. This patient’s progressive neurologic symptoms warranted aggressive chemotherapy. Further study is needed to determine optimal therapy.

(POSTER 227) FEASIBILITY AND PARENT SATISFACTION OF A PHYSICAL THERAPY INTERVENTION PROGRAM FOR CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA IN THE FIRST SIX MONTHS OF MEDICAL TREATMENT

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Background: Children with acute lymphoblastic leukemia (ALL) are at risk for developing neuromuscular and musculoskeletal complications during and after their medical treatment. However, it is suggested that if children participate in an individualized physical therapy exercise program, these limitations may be prevented or at least reduced.

Objectives: The objective of this study was to examine the feasibility of an in-hospital physical therapy- and home exercise program for children with newly diagnosed ALL during the first four phases of medical treatment, when the medical treatment is most aggressive.

Design/Method: Nine patients, between the ages of 2 to 14 years old were enrolled at diagnosis in the study. Each patient was evaluated at diagnosis, after each cycle of therapy (the first four phases) and each time patients were re-admitted to the hospital. Following the initial physical therapy evaluation an individualized home exercise program was developed, consisting of stretching, strengthening, and aerobic exercises. The following end points were measured at each evaluation: gross motor assessment as measured by Gross Motor Function Measure (GMFM), health related quality of life as measured by the PedoQL and parent satisfaction questionnaire.

Results: This study was feasible with 98% of the evaluation sessions completed. The GMFM and PedoQL improved steadily throughout the study; however the PedoQL slightly decreased from interim maintenance to delayed intensification. The parents reported being satisfied with the physical therapy.

Conclusion: We demonstrated that an in-hospital- and home exercise physical therapy program during the first four phases of medical treatments is feasible. Future randomized studies are needed to confirm whether initiating a physical therapy program at diagnosis in children with ALL will limit functional impairment, improve overall fitness and increase health-related quality-of-life.

(POSTER 228) MAXIMIZING THE USE OF TMP-SMZ FOR PCP PROPHYLAXIS IN PEDIATRIC ONCOLOGY PATIENTS

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Background: Trimethoprim/Sulfamethoxazole (TMP-SMZ) is the most effective agent for Pneumocystis carinii (PCP) prophylaxis in immunocompromised patients (1). Recently, it has been shown that TMP-SMZ at lower and less frequent doses provide effective prophylaxis with minimal, if any, myelosuppression (2).

Objectives: We undertook to maximize the percentage of our pediatric oncology patients who receive TMP-SMZ at the minimum appropriate dose for PCP prophylaxis.

Design/Method: We reviewed the charts of 100 pediatric oncology patients on chemotherapy to determine the current PCP prophylaxis medication and dose. We updated our program guideline for PCP prophylaxis to recommend TMP-SMZ at a dosage of 5 mg/kg (maximum 160 mg) po daily 3 days/week. Other agents were only recommended for patients with allergy to TMP-SMZ. Myelosuppression was no longer considered an acceptable reason to stop TMP-SMZ. We also implemented a monthly educational campaign which included updates at staff meetings and conferences, patient/family handouts, posted guidelines in all clinical works spaces, and changes to our online formulary and computerized orders. Two months after the educational intervention, we re-evaluated the original 100 patients and any new patients who had been started on chemotherapy to determine the percentage who were now on TMP-SMZ, at the new lower recommended dose for PCP prophylaxis.

Results: Of the original 100 patients, the percentage of eligible patients on TMP-SMZ rose from 79.8% to 90.4% and the percentage of patients on the newer recommended dose increased from 61.2% to 92.4%. Of the 39 newly diagnosed patients, 95.8% were on TMP-SMZ for PCP prophylaxis and 92.3% of these patients were on the correct dose.

Conclusion: We conclude that a targeted education program directed at patients and providers is successful in increasing the percentage of eligible patients who are on TMP-SMZ for PCP prophylaxis at an appropriate dose. Furthermore, modifications to our systems—including posted signs, updated formularies, and standardized computer order entry—as well as to our prescribing culture are helpful in maintaining these important changes.

(1) Vasconcelles et al, Bip Blood Marrow Transplant, 2000
(2) Lindenmuller and Albano, Pediatrics, 2007

(POSTER 229) EHRICHIA-INDUCED HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN TWO CHILDREN

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Background: Hemophagocytic lymphohistiocytosis (HLH) is a familial or acquired disease in which there is uncontrolled activation of the cellular immune system. HLH is a potentially life-threatening condition with a fulminant clinical course involving irreversible tissue damage and multi-organ failure. The disease is often rapidly fatal without prompt diagnosis and treatment. Secondary HLH is a reactive process resulting from an insult such as a malignancy, immunodeficiency, autoimmune disease, or, more commonly, an infection. A wide range of infectious agents have been associated with HLH. Ehrlichiosis, a tick-borne disease endemic to the eastern United States, is a rare trigger of HLH. We present the first two reported cases of ehrlichiosis-associated hemophagocytic lymphohistiocytosis in children.

Objectives: To demonstrate the importance of considering Ehrlichiosis as a possible trigger of HLH.

Design/Method: A review of the literature and patient record.

Results: CASE 1: A 10 year old female presented with a history of fever, rash, and bone pain. She had a diffuse rash over her chest, arms, and abdomen. Labs revealed pancytopenia, elevated ferritin, and coagulopathy. She was hypotensive and hypoxic. Antibiotic therapy was started with doxycycline and cefepime. Ehrlichia titters were positive. A bone marrow biopsy revealed hemophagocytosis. sL1-2 receptor and performin were abnormal. A diagnosis of HLH was made and steroids were started. The patient recovered. CASE 2: A 13 year old male presented with a one week history of fever, headaches, and fatigue. He developed acute respiratory distress and was intubated. He had pancytopenia with high ferritin and triglycerides and a coagulopathy. Titters for E. chaffeensis were positive. Triple antibiotics plus doxycycline were started. A bone marrow aspirate and biopsy revealed hemophagocytosis. The sL1-2 receptor was elevated. A diagnosis of HLH was made. Therapy was started with dexamethasone and cyclosporine and the patient rapidly...
improved. He was discharged and completed an eight week course of dexamethasone, cyclosporine, and etoposide.

Conclusion: Ehrlichiosis is a rare trigger for HLH, but should be considered when patients in an endemic region present with HLH. Appropriate treatment for HLH and ehrlichiosis should be initiated as soon as possible as the disease can be rapidly fatal.

(POSTER 230) EPIEMIOLOGIC MAPPING OF FLORIDA CHILDHOOD CANCER CLUSTERS: AN UPDATE

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Background: Childhood cancer remains the leading cause of disease-related mortality for children. Whereas improvement in care has dramatically increased survival, the risk factors remain to be fully understood. The increasing incidence of childhood cancer in Florida may be associated with possible cancer clusters.

Objectives: We aimed, in this study, to identify and confirm possible childhood cancer clusters and their subtypes in the state of Florida.

Design/Method: We conducted purely spatial and space-time analyses to assess any evidence of childhood malignancy clusters in the state of Florida using SaTScanTM. Data from the Florida Association of Pediatric Tumor Programs (FAPTP) for the period 2000-2008 were used in this analysis.

Results: In the purely spatial analysis, the relative risks (RR) of overall childhood cancer persisted after controlling for confounding factors in south Florida (SF) (RR, 1.36, p = 0.001) and northeastern Florida (NEF) (RR = 1.30, p = 0.01). Likewise, in the space-time analysis, there was a statistically significant increase in cancer rates in SF (RR, 1.52, p = 0.001) between 2006 and 2007. In 2008, the SF cluster persisted (RR = 1.38, p = 0.001). The purely spatial analysis of the cancer subtypes indicated a statistically significant increase in the rate of leukemia and brain/CNS cancers in both SF and NEF, p < 0.05. The space-time analysis indicated a statistically significant sizable increase in brain/CNS tumors, (RR 2.25, p = 0.02) for 2006-2007.

Conclusion: There is evidence of spatial and space-time childhood cancer clustering in south and northeastern Florida. Preliminary evidence suggests that the clustering remains present in 2008. This evidence is suggestive of the presence of possible predisposing factors in these cluster regions.

(POSTER 231) CAPNOCYTOPHAGA SPECIES ASSOCIATED MORTALITY IN A CHILD WITH AML

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Background: Capnocytophaga species is a slow-growing gram negative bacteria commonly found in canine saliva. It has been associated with fulminate septicaemia in immunocompetent and immunocompromised hosts, with mortality rates being as high as 30%. Infection is characterized by fever, abdominal pain and rigors and progresses to multi-system organ failure. Literature reports Capnocytophaga infection more commonly in the immunocompromised host, as asplenic patients appear to have increased susceptibility to the bacteria. Such associated sepsis in the pediatric oncology population has been scarcely reported, and to our knowledge no such cases reported in neutropenic patients.

Objectives: We report a case of Capnocytophaga-septicaemia in a neutropenic patient undergoing induction chemotherapy for AML.

Design/Method: We present a young female new diagnosed with AML who experienced fever, abdominal pain, and eventual cardiogenic shock as a result of concurrent enterococcus and Capnocytophaga septicaemia. Unfortunately, the patient expired in spite of aggressive antibiotic therapy.

Results: A 12 year-old female newly diagnosed with AML presented for induction chemotherapy. The patient completed treatment per protocol and subsequently became severely neutropenic with an ANC of zero. On day 16 of her hospital stay, the patient developed fever of 102.5, for which cefepime, vancomycin, meropenem and tobramycin were added to her regimen. She experienced diffuse, cramping abdominal pain along with hypertension, tachycardia and tachypnea which necessitated transfer to the ICU. Two blood cultures were drawn at onset of fever; the first revealed Enterococcus faecium. Three days later, the second blood culture resulted positive for Capnocytophaga species. Infectious disease service was consulted and patient maintained on the current antibiotic regimen. However, despite aggressive antibiotic therapy, she developed respiratory failure which required intubation. She experienced fevers as high as 106°F and was maintained on ionotropic agents. Unfortunately, she continued to deteriorate and developed cardiogenic shock and eventual multi-organ failure. Palliative care was initiated and, four days after the onset of symptoms, the patient expired.

Conclusion: Capnocytophaga is a slow growing bacteria that necessitates aggressive antibiotic therapy to prevent morbidity and mortality. The neutropenic patient should be considered high risk for infection with this organism. Exposure risks may also need to be considered in these patients.

(POSTER 232) USING TARGETED POOLED SAMPLE DNA SEQUENCING TO IDENTIFY RARE GENETIC VARIANTS ASSOCIATED WITH HIGH-RISK PEDIATRIC ALL.

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Background: Acute lymphoblastic leukemia (ALL) is the most common childhood cancer. Despite much research, the etiology of this complex disease remains unknown. Mounting evidence suggests that much of the variability observed in complex disease is due to inter-individual rare variants. While a particular variant may be rare, a critical gene may harbor multiple variants in an affected cohort resulting in the same phenotype. Identifying rare causal variants at multiple loci requires deep resequencing from a population of affected individuals. This strategy was recently proven successful on a smaller scale using traditional Sanger sequencing studying IKZF1 copy number alterations(1) and JAK mutations(2) in high-risk ALL. However, Sanger sequencing is too cost and time prohibitive for large-scale analyses.

Objectives: In collaboration with the Children’s Oncology Group, we will resequence 56 genes in the germline DNA of unaffected children as well as germline and leukemia DNA from 96 pediatric high-risk leukemia patients treated on PO9906 to identify genes harboring an excess of rare variants in patient DNA compared to unaffected DNA.

Design/Method: We have designed a pooled DNA sequencing method to leverage the high-throughput capacity of next-generation sequencing.(3) We have selected 43 genes, all significant by genome-wide array analysis in these same patients(4), as well as 13 other genes in the same metabolic pathways for resequencing. I will compare matched patient germline and leukemia DNA with similar pooled sequencing in an unaffected pediatric cohort. We expect to identify many loci in ALL patients demonstrating a higher degree of genetic variation compared to the unaffected cohort and thus potentially leukemogenic. We will validate these results in 250 pediatric high-risk ALL patients treated on ALL0232. Sequencing will be followed by individual genotyping of validated loci to identify potential inter-individual gene-gene interactions.

Results: Pooled sequencing is currently in progress. We expect to identify a variety of genes and pathways involved in pediatric leukemogenesis.

Conclusion: Validated loci will inform long-term functional studies with the ultimate goal of providing prescriptive individual patient genotyping for risk assessment and targeted therapy.

References
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(POSTER 233)�THIRTY DAY READMISSION RATES FOLLOWING HOSPITALIZATION FOR SICKLE CELL ANEMIA CRISIS AT FREESTANDING CHILDREN’S HOSPITALS

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Background: The National Association of Children’s Hospitals (NACHRI) proposed using 30-day readmission rates for sickle cell crisis as a quality marker, although there is limited data on factors leading to readmission.

Objectives: To describe the 30-day readmission rates following sickle crisis among children’s hospitals in a national database, identify factors associated with readmission, and describe hospital variation in readmission rates.

Design/Method: We performed a retrospective examination of 12,216 hospitalizations for sickle cell crisis from 7/1/2006–12/31/2008 at 37 freestanding children’s hospitals in the Pediatric Health Information System (PHIS) database. Hospitalizations were limited to NACHRI criteria (age < 18, APR DRG code 662 (sickle cell crisis), discharged home, length of stay < 2 SD from the mean). Using multivariate modeling with generalized estimating equations to account for repeated
Results: The 12,216 qualifying hospitalizations represented 4829 patients, with 1–30 admission per patient (49% of patients had one admission). 2880 hospitalizations (17%) resulted in readmission within 30 days (range 0-30, mean 14). 332 readmissions (16%) occurred within 3 days and 669 (32%) within 7 days of discharge. Factors significantly associated with readmission were age (OR 1.15; 95% CI 1.1–1.2), HbSS genotype (OR 1.8, 95% CI 1.0–3.3), simple transfusion (OR 0.31, 95% CI 0.16–0.61), acute chest syndrome (OR 0.40, 95% CI 0.22–0.75) and census region (1 OR 0.41, 95% CI 0.19–0.88, region 2 OR 0.56, 95% CI 0.34–0.93, region 3 OR 0.36, 95% CI 0.22–0.58, region 4 reference). Adjusted hospital readmission rates ranged from 0-24% with little variation.

Conclusion: Approximately 17% of hospitalizations for sickle crisis among pediatric patients is in a sample of free-standing children’s hospitals are followed by readmission within 30 days. Older patients, those with HbSS, and those admitted for pain are more likely to be readmitted; simple transfusion was protective. These findings suggest that more multi-institution studies are needed before a 30-day readmission rate is widely adopted as a quality marker.

(PAPER 234) REDEFINING CHRONIC HEMOLYSIS IN PEDIATRIC SICKLE CELL DISEASE: FOCUS ON SYMPTOMATIC CHOLELITHIASIS

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Background: Previously, the morbidity/mortality of sickle cell disease (SCD) was assumed to be solely related to vascular occlusion by red cell sickling; current evidence suggests that chronic hemolysis secondary to endothelial dysfunction and vascular injury plays a significant role. Early giant cell medullary disease has yet to be established as another marker of sickle hemolysis in pediatric SCD. Therefore, it is essential to better understand the relationship between gallstone development and other SCD complications related to chronic hemolysis.

Objectives: The primary objective was to perform a retrospective review of all patients with SCD who underwent cholecystectomy in order to elucidate degree of hemolysis, frequency of SCD complications, determine inciting factors, and compare those to age-matched controls.

Design/Method: This study was conducted at The Children’s Hospital of Philadelphia. Case recruitment included all pediatric patients with SCD, type SS or S/Sjthal, who underwent cholecystectomy. Controls were age-matched patients with SS or S/Sjthal who had not undergone cholecystectomy. Patient records were reviewed for clinical data.

Results: Overall, 138 SS patients and 4 S/Sjthal patients underwent cholecystectomy from January 1993 to November 2008. Mean age at cholecystectomy was 12.9 years and the primary presenting symptom was abdominal pain. 15% of cases had frank cholecystitis. Post-surgery, 70% had no major complications. We found a statistically significant difference in the occurrence of stroke. 14.1% of cases had a stroke versus 3.9% of controls (p = 0.0142). Additionally, 14.5% of cases had at least one abnormal TCD versus 5.7% of controls, with borderline significance (p = 0.0552). The average number of TCD velocities for cases was 5.1 versus 2.6 for controls (p < 0.0001). Regarding VO2 treatment, 9.4% of cases received patient-controlled analgesia during at least one admission compared to 6.6% of controls (p = 0.0007). Total white blood cell count was significantly different between cases and controls while controlling for age (p = 0.0388).

Conclusion: Patients who underwent cholecystectomy had an increased incidence of stroke and a higher number of VO2 admissions. Most patients 3–5 years of age at the time of cholecystectomy had increased SCD complications compared to controls.

(PAPER 235) SICKLE RED BLOOD CELLS HAVE INCREASED PHOSPHORYLATION OF ADDUCIN AND INCREASED REACTIVE OXYGEN SPECIES PRODUCTION MEDITATED BY SIGNALING PATHWAYS INVOLVING PKC AND Rac GTases

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Background: Sickle hemoglobin (HbSS) polymerization in hypoxic conditions is the trigger for sickling deformation of red blood cells (RBC) containing HbSS, but the mechanisms by which reversibly sickled cells become irreversibly malformed are poorly understood. The small Rho GTases Rac1 and Rac2 regulate actin structures, a component of the cytoskeleton, in conjunction with PKC. Deficiency of Rac1/2 GTases in mice disrupts the RBC cytoskeleton and reduces erythrocyte deformability. Additionally, Rac GTases induce reactive oxygen species (ROS) production via NADPH oxidase in several cell types.

Objectives: We hypothesized that alterations in PKC and Rac GTase activity in sickle RBCs result in cytoskeletal disruption and increased ROS-mediated cell damage, thus contributing to morphological and functional abnormalities in these cells.

Design/Method: We evaluated cytoskeletal phosphorylation changes in RBCs from patients with HbSS and from control subjects with hemoglobin AA (HbAA). We also examined the subcellular localization of adducin and active Rac GTase in normal and sickle RBCs and analyzed ROS production in both cell types in the absence and presence of PKC and Rac GTase activators and inhibitors.

Results: Phosphorylation of adducin and Band 4.1 is significantly increased in the erythrocyte cytoskeleton of HbSS RBCs. Activation of PKC or inhibition of Rac result in increased phosphorylation of adducin while PKC inhibition results in decreased phosphorylation of adducin in RBCs. The subcellular distribution of active Rac GTase is also altered in sickle RBCs relative to AA RBCs. ROS generation is elevated in HbSS-RBCs by 150-250% compared to that in HbAA-RBC and a significant portion of this is due to NADPH oxidase activity. Inhibition of PKC or Rac activity by small molecule inhibitors results in decreased ROS production, while PKC activation increases ROS production. Rac also appears to acts downstream of PKC in ROS production. Finally, we demonstrate that the induction of enzymatic ROS production in sickle RBCs requires free intracellular calcium.

Conclusion: Our results implicate PKC and Rac in aberrant cytoskeletal protein phosphorylation and increased ROS production in sickle RBCs. These alterations may contribute to membrane changes induced during cell sickling. Elucidation of these pathways may identify new therapeutic targets in sickle cell disease.

(PAPER 236) CEREBRAL TISSUE HEMOGLOBIN DESATURATION IN CHILDREN WITH SICKLE CELL DISEASE

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Background: Hemoglobin (Hb) desaturation is a common finding in sickle cell disease (SCD) that is associated with abnormally increased cerebral arterial blood flow velocities and overt stroke. Little is known about Hb saturation in cerebral tissue in SCD.

Objectives: We aimed to describe the distribution of absolute cerebral tissue Hb saturation (Sc(t)O2) in children with SCD and identify relationships between Sc(t)O2 and: peripheral Hb saturation measured by pulse oximetry (SpO2), age, SCD genotype, steady-state hematogetic values, and transcranial Doppler ultrasonography (TCD) velocities.

Design/Method: We used transcutaneous near-infrared spectrophotometry (CASMED FORE-SIGHT®) with bi-frontal probes to measure absolute Sc(t)O2 in children with SCD during steady-state clinic visits. Measurements take < 5 minutes. Pearson correlation and multiple linear regression were used. Sample size was calculated to detect a minimum correlation (r) of 0.3 between Sc(t)O2 and SpO2 (r = 0.05, 1–β = 0.8).

Results: We studied 80 children (60 SS/Sj0; 20 SC/Sj0). Sc(t)O2 is known to be 65-80% in normoxic individuals breathing room air. Sc(t)O2 was skewed markedly lower in SCD patients. Mean Sc(t)O2 was 55.5 ± 14.3% (mean ± SD) in SS/Sj0 and 66.7 ± 12.7% in SC/Sj+ patients. Sc(t)O2 was abnormally low (< 65%) in approximately 75% of SS/Sj0 and 25% of SC/Sj+ patients. In bivariate analyses, Sc(t)O2 correlated significantly with age (r = −0.43; P < 0.001), Hb concentration (r = 0.45; P < 0.001), reticulocyte count (r = 0.43; P < 0.001), and SpO2 (r = 0.36; P = 0.003), but not TCD velocities. In multivariable models, the two significant determinants of right-sided Sc(t)O2 were SpO2 (P < 0.001) and TCD velocity in the right anterior cerebral artery (P = 0.027); the only significant determinant of left-sided Sc(t)O2 was SpO2 (P = 0.008). In patients with marked right-left asymmetry for Sc(t)O2, TCD velocities were higher on the side with lower Sc(t)O2.

Conclusion: Cerebral Hb desaturation is common in SCD, more severe in SS/Sj0 patients, and associated with peripheral Hb desaturation and increased TCD velocity in the anterior circulation. Cerebral tissue desaturation, which can be detected by a rapid, non-invasive technique, may be a mechanistic link between peripheral Hb desaturation and stroke.

(PAPER 237) DECREASE IN MICROVASCULAR BLOOD FLOW IN SICKLE CELL ANEMIA TRIGGERED BY AUTONOMIC RESPONSES TO SIGHING

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Background: Sickle cell anemia (SCA) is a genetic disorder characterized by recurring episodes of vasoocclusive crisis (VOC) that can lead to hospitalization or sudden death.

Objectives: To better understand the pulmonary mechanisms leading to VOC, we studied the physiologic responses to transient hypoxia induced in subjects with SCD.

Design/Method: Tidal volume (VT), arterial oxygen saturation, electrocardiogram (ECG), and microvascular perfusion (PU) by laser-Doppler were continuously recorded during each 40-minute experimental session. Heart rate variability (HRV), which is an accepted index of the sympathetic/parasympathetic balance of the autonomic nervous system (ANS), was computed from the ECG signal.

Results: We observed multiple prominent drops in PU in SCA subjects (n = 8) that were not as clearly evident in controls (CTL; n = 9). We examined tidal volume tracings obtained simultaneously with perfusion data: we observed that PU drops frequently followed sighs in SCA subjects but rarely in CTL. PU drops were associated with sighs in 8 of 8 SCA patients and in 6 of 9 CTL subjects (p < 0.05, Chi-square). The likelihood ratio of sigh-associated PU drops was significantly higher in SCA than CTL subjects (median = 77.78% vs. 14.29% for SCA vs. CTL, p = 0.0088, rank-sum test), thus indicating that SCA patients are much more likely to have sigh-associated peripheral vasoconstriction. Analysis of HRV showed a substantial increase of sympathetic modulation in response to sighs in SCA relative to CTL subjects (p < 0.05), indicating a marked abnormality of the ANS in SCA.

Conclusion: The likelihood of coupling between spontaneous sighs and subsequent vasoconstrictive events (i.e., PU drops) is much higher in SCA patients than in CTL. Since the sigh-vasoconstrictor response is controlled by the ANS, we speculate that a drop in perfusion secondary to increased neuronal coupling between the lung and vasculature may be an initiating event in VOC. In a background of HBO2 transient decreases in perfusion may prolong red cell residence time in the microvasculature, leading to HBO2 polymerization, sickling and vascular occlusion.

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Background: The Stroke Prevention Trial in Sickle Cell Anemia (STOP) showed that children with sickle cell anemia at high risk of stroke could be identified by transcranial Doppler ultrasonography and incident strokes could be prevented by chronic erythrocyte transfusions. Although this approach to primary stroke prevention (PSP) appears to be efficacious, its effectiveness outside the clinical-trial setting is unknown on a national scale.

Objectives: We sought to describe the effectiveness of PSP in a national sample since the 1998 publication of the STOP trial.

Design/Method: We performed a trend analysis of the 1994-2007 National Inpatient Sample discharge databases which constitute a 20% stratified sample of all US hospitalizations and are weighted to allow national estimation. We identified discharges with any sickle hemoglobinopathy (SCD) by ICD-9CM code. Within SCD discharges, acute strokes were identified using an ICD-9CM search strategy with a published sensitivity of 86% and specificity of 95%.

Results: For the 1335 discharges for stroke from 1994-2007, the median age was 8 years; the case-fatality rate was 3.3%. US rates of hospitalization for stroke decreased since the STOP trial’s publication (P < 0.0001; Figure). In the 5 years (1994–1998) before the STOP trial’s publication, the estimated mean stroke hospitalization rate was 127.8/year (95% confidence interval: 105.5–150.8), compared with 84.8/year (95% C.I.: 61.1–108.5) for 1999–2003 and 68/year (95% C.I.: 48.8–87.2) for 2004–2007.

Conclusion: Stroke rates have declined for children with SCD since the introduction of PSP, although substantially less than previously reported.

Tables/Charts:
samples for 36/50 patients. Serum MIF levels were measured by ELISA (R D Systems, MN, USA).

Results: The association between MIF levels at 24hrs of admission and VOC and ACS was examined using two-way analysis of variance (ANOVA). Serum MIF levels at 24 hours after admission were significantly higher in SCD patients who developed ACS (N = 10/36, Mean = 4989 pg/ml, range: 114-13515 pg/ml) compared to patients without ACS (N = 26/36, Mean = 1886.9 pg/ml, range: 44-5964 pg/ml) (p < 0.006). Serum MIF levels did not differ in patients with VOC only (n = 16, mean = 3333.81 pg/ml, range: 112-13515), fever only (n = 12, mean = 2871.42 pg/ml, range 44-10075) or fever and VOC (n = 8, mean = 3394.25 pg/ml, range: 114-11949 pg/ml). Further analysis of other timed specimens is in progress.

Conclusion: Our pilot study demonstrates that elevated serum MIF levels are associated with the development of ACS in SCD patients. The observation of the association of elevated levels of MIF and ACS should lead to studies that explore the possible role of MIF in the pathophysiology of ACS. MIF should be explored as a potential therapeutic target in patients with ACS.

(PAPER 241)
NEUROCOGNITIVE FUNCTIONING AND QUALITY OF LIFE AMONG CHILDREN WITH SICKLE CELL ANEMIA AND STROKE
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Background: Stroke is a devastating complication that affects 5-10% of children with sickle cell anemia (SCA), and often is associated with deficits in neurocognitive and psychosocial functioning that may compromise quality of life (QOL).

Objectives: To determine prospectively the neurocognitive deficits and QOL in a large cohort of children with SCA and documented clinical stroke who receive chronic transfusions.

Design/Method: Pediatric patients with SCA and previous stroke on monthly transfusions were eligible for enrollment in the multicenter Stroke With Transfusions Changing To Hydroxyurea (SWITCH, NCT00122980) clinical trial. All enrolled subjects had their previous index stroke documented by central blinded review, and completed baseline tests including standardized neurocognitive testing and QOL assessments using the PedsQL and CHQ-50 questionnaire instruments.

Results: A total of 161 subjects were enrolled from 25 clinical sites (mean age 12.9 ± 4.0 years, range 5.0-19.0 years). Almost all had HbSS and were African American, non-Hispanic with a Male/Female ratio of 83/77. For 137 subjects who completed some baseline neurocognitive testing, achievement scores were well below the normative mean of 100 ± 15 for both broad reading (77.4 ± 22.1) and math (75.4 ± 21.2). Cognitive abilities were also below the normative mean in general intellectual ability (78.2 ± 17.0), verbal ability (82.2 ± 15.3), processing speed (75.2 ± 20.8), working memory (82.6 ± 18.0), broad attention (78.8 ± 18.6), and executive processes (82.0 ± 15.5). Health-related QOL included low values on the CHQ for both the physical and psychosocial summary scores, but above values previously reported for children with mild or severe SCA. With the PedsQL instrument, subjects rated themselves below healthy norms in all subscales, but higher than their parent/caregiver’s proxy ratings for physical functioning, social functioning, and school functioning.

Conclusion: In SWITCH, children with SCA and documented clinical stroke have significant neurocognitive deficits in all tested areas. QOL is also below normal but somewhat higher than predicted for children with SCA, probably due to transfusion therapy. Subsequent analyses will evaluate neurocognitive and disease predictors of QOL outcomes.

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(PAPER 242)
EVALUATION OF CHILDREN WITH SICKLE CELL DISEASE TREATED FOR CHRONIC OR RECURRENT PAIN USING A MULTIDIMENSIONAL PAIN MANAGEMENT MODEL
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Background: Pain in sickle cell disease (SCD) is acute or chronic and leads to school absenteeism, impaired health-related quality of life and early mortality.

Objectives: Given the little known about characteristics and health care utilization of children with SCD and chronic pain, we sought to 1) describe characteristics of children with chronic or recurrent pain and 2) evaluate the impact of a multidimensional pain management model (involvement of pain physician, pain psychologist, social worker with team/child) on pain hospitalizations.

Design/Method: We conducted a retrospective cohort study of children with SCD ages 2-18 referred to our institution’s pain clinic between 1999-2008. Referrals happen when requirements develop for chronic narcotics and/or frequent pain hospitalizations occur. Descriptive statistics evaluated patient characteristics and Wilcoxon Signed Rank evaluated the change in number of pain hospitalizations one year before and after referral.

Results: Median age of the 19 children identified was 15 (IQR 11-17), significantly more were female (78.9% vs. 21.1%; p = 0.012) and majority (73.7%) had HbSS disease. All reported taking narcotics at time of referral, 68.4% were receiving hydroxyurea; half of those not on hydroxyurea started it upon referral (n = 3). No children were chronically transfused before referral; one initiated transfusions upon referral. Only 5/26.3% had avascular necrosis; thus significantly more children did not have a pathologic finding to explain their pain other than SCD (26.3% vs. 73.7%; p = 0.04). Almost all (89.5%) learned non-pharmacologic pain management techniques. The median number of pain hospitalizations significantly decreased after referral for the entire cohort [5 (IQR 3-6) to 1 (IQR 0-4); p = 0.0006]. To further delineate the pain clinic’s effect, analysis was repeated after removing 4 children started on hydroxyurea/chronic transfusions upon referral. The significant decrease in median hospitalizations persisted [5 (IQR 3-6) to 1 (IQR 0-4); p = 0.022].

Conclusion: A multidimensional pain management model effectively decreased pain hospitalizations. Majority of children did not have avascular necrosis to explain their chronic pain, suggesting potential abnormal pain neurobiological mechanisms. Targeting high risk children early using a multidimensional pain management model may improve outcomes for children with SCD.

(PAPER 243)
NEOPLASIA IN DIAMOND BLACKFAN ANEMIA (DBA) AS REPORTED TO THE DIAMOND BLACKFAN ANEMIA REGISTRY (DBAR)
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Background: DBA is one of the rare, inherited bone marrow failure syndromes and is characterized by red cell aplasia, congenital anomalies and a predisposition to cancer. Cancer and myelodysplastic syndrome in DBA patients have been reported in the literature, with a predominance of hematologic malignancies; osteogenic sarcoma was reported by the DBAR and other registries.

Objectives: Our purpose was to report the types of neoplasia, the age of onset and the outcome in DBA patients enrolled in the DBAR.

Design/Method: The DBAR is a comprehensive database of patients with DBA in North America who are enrolled after informed consent is obtained. The patients, their families, and their physicians completed a detailed questionnaire. A review of medical records and telephone interviews were performed to clarify and verify the information.

Results: As of December 31, 2009, 583 patients were enrolled in the DBAR, with 13 patients reporting 15 neoplasms (12 patients had one neoplasm, 1 patient had 3); the crude rate was 2.2%. Eight of these were previously reported by the DBAR and one was also reported in the literature (Gri, BJH 2000). There were 6 males and 7 females. The median age at diagnosis of neoplasia was 43 years (range, 4 to 69 yrs). There were 3 patients with myelodysplastic syndrome and one with acute myeloid leukemia (AML). Solid tumors included osteogenic sarcoma (3 patients), colon cancer (2 patients), soft tissue sarcoma (1 patient), squamous cell carcinoma (2 patients), uterine cancer (1 patient) and breast cancer (2 patients). Three patients were alive and 10 died from progressive disease and/or therapy-induced toxicity. Eight patients were transfusion-dependent at the time of their diagnosis; 2 patients were steroid-dependent; 1 was in remission; 1 patient had a known DBA mutation (RPS19) but was never diagnosed. Genotyping was available for 7/13 patients; 5/7 had an identifiable mutation (3/5 RPS19, 2/5 RPS11).

Conclusion: DBA is a cancer predisposition syndrome. Although not statistically verified, it appears as if cancer portends a poor outcome in most patients. The age at diagnosis of the neoplasia appears to be younger than expected. Genotype-phenotype correlations are not yet evident.

(PAPER 244)
ERYTHROCYTE ALLO- AND AUTO-ANTIBODY FORMATION IN SICKLE CELL ANEMIA DURING STROKE PROPHYLAXIS

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Background: Red blood cell (RBC) allo- and auto-antibody formation frequently occurs among chronically transfused patients with sickle cell anemia (SCA). Extended RBC phenotype matching for Cc, Ee, Kk, and Rh(D) (CDEK matching) has long been advocated for patients on chronic transfusion therapy, but this recommendation has an unclear benefit in actual clinical practice.

Objectives: To determine the prevalence and types of RBC allo- and auto-antibodies found in children with SCA and stroke, who are receiving monthly transfusions for secondary stroke prophylaxis in an era of extended RBC phenotype matching.

Design/Method: All subjects were enrolled in the Stroke With Transfusions Changing to Hydroxyurea (SWiTCH) study. During study intake, detailed information was gathered about previous RBC allo- and auto-antibody formation.

Results: A total of 161 subjects were enrolled in SWiTCH; almost all were African-American institutions across the US, all of which routinely perform extended RBC phenotype matching for children with SCA. During study intake, detailed information was gathered about previous RBC allo- and auto-antibody formation.

Conclusion: In the multicenter SWiTCH trial, children with SCA on chronic transfusions for secondary stroke prophylaxis entered the study with a high frequency of RBC allo-antibody formation, especially against C, E, and Kell antigens. These RBC allo-antibodies developed despite most transfusions being given in an era where extended RBC phenotype matching for CDEK antigens is recommended and typically available.

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(POSTER 245)
IRON-INDUCED HEMOLYSIS IN PRETERM INFANTS

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Background: For pre-term infants, the physiologic nadir of hemoglobin usually occurs before 12 weeks of age and can be quite significant, in an inverse relationship with birth weight and gestational age. Their iron stores also become more quickly depleted than in term infants as they undergo catch-up growth. Nearly 40 years ago, studies in premature infants found hemolytic anemia occurred in vitamin E deficient infants given high doses of enteral iron. Commercial formulas have since been adjusted to prevent vitamin E deficiency. Current practice is to start iron supplementation at 2-4 mg/kg/day in preterm infants once they are on full feeds. Many pediatric intensive care units give high doses of enteral iron. Some commercial formulas have since been adjusted to prevent vitamin E deficiency. Current practice is to start iron supplementation at 2-4 mg/kg/day in preterm infants once they are on full feeds.

Objectives: Younger and smaller premature infants are surviving with less morbidity than previously. Here we report a case series of premature infants who developed iron-induced hemolytic anemia.

Design/Method: We report five pre-term infants managed per standard practice at the Children’s Hospitals and Clinics of Minnesota-Minneapolis between October 2007 and May 2009. The average gestational age at birth was 27.7 weeks (27-28 weeks), and average birth weight was 996 grams (760-1240 grams). The average birth hemocrit was 45.4 g/dL. At a mean of 19 days (11-27 days), oral iron was started at 2-4 mg/kg/day. Results: Hemolytic anemia was noted a mean of 14 days later (8-32 days). Hemocrit drops averaged 11.4% (3.8-21.7%). Coomb’s tests and newborn screens were negative. Heinz bodies were found in the 4 patients tested with a mean of 20.9% (14.6-43.4%). Two patients required transfusions. The three non-transfused patients demonstrated elevated hemoglobin F levels from 88-93% (mean 90.6%). Hemocrits improved gradually after discontinuation of the iron in all patients.

Conclusion: All of the patients were very low birth weight and all received supplemental iron before 1 month of age. We postulate that hemoglobin F is more susceptible to oxidative damage by oral iron. As more very low birth weight infants are surviving with fewer complications, feeds are being initiated earlier. Many of these infants do not receive transfusions, thus have high hemoglobin F levels. Close observation for hemolysis is required around the time of iron initiation in these neonates.

(POSTER 246)
ASSOCIATION BETWEEN HYDROXYUREA AND HEALTH-RELATED QUALITY OF LIFE IN CHILDREN WITH SICKLE CELL DISEASE

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Background: Hydroxyurea is used in children with sickle cell disease (SCD) to prevent acute and chronic complications. It is unclear how hydroxyurea impacts disease-related quality of life (HRQL).

Objectives: Our goal was to test whether children taking hydroxyurea have higher HRQL scores than children not taking hydroxyurea.

Design/Method: We conducted a two-institution retrospective cross-sectional study of children with SCD not on chronic transfusion therapy who had previously completed the PedsQL 4.0. Data were abstracted from the medical record regarding genotype, disease severity, and hydroxyurea therapy and PedsQL scores. Chi-square and Wilcoxon tests were done to compare categorical and continuous variables respectively. All HRQL comparisons were adjusted for age, gender, genotype and disease severity.

Results: One hundred ninety-one children (mean age 10.4 ± 4.7 years; 51% males; 99% Black/African American) were included. At the time of HRQL assessment 114 had been taking hydroxyurea for 48.7 ± 31.4 months. Compared to children in the no hydroxyurea group (n = 77), children in the hydroxyurea group were older (p < 0.001) and more had the SS genotype (p < 0.001) and severe disease (p < 0.001). Children in the hydroxyurea group had higher self-report total PedsQL median [IQR] scores than children in the no hydroxyurea group (hydroxyurea group 75 [62.0, 86.4], no hydroxyurea group 69.0 [54.1, 79.9]; p = 0.04). Child self-report physical functioning scores were significantly higher for children on hydroxyurea (hydroxyurea group 79.7 [62.5, 90.6], no hydroxyurea group 71.4 [58.6, 81.2]); p = 0.001). Similarly, parent-proxy physical functioning scores were significantly higher for children on hydroxyurea (hydroxyurea group 75 [53.9, 87.5], no hydroxyurea group 71.9 [53.2, 90.6]; p = 0.05).

Conclusion: Although children with SCD taking hydroxyurea have more severe disease, their HRQL was better than children in the no hydroxyurea group particularly in the physical functioning domain. Future studies should use cell specific HRQL instruments to detect the benefit of hydroxyurea on the HRQL of children with SCD.

(POSTER 247)
PROGNOSTIC VALUE OF Pro-BNP AND VEGF IN CHILDREN WITH SICKLE CELL DISEASE

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Background: Sickle cell disease (SCD) is a chronic hematologic disease associated with life-long morbidities including pain crises, acute chest syndrome, chronic organ damage, and a decreased life expectancy. Markers of chronic hemolysis, severe anemia, leukocytosis, and thrombocytosis have repeatedly been shown to correlate with an overall worse outcome. Pro-BNP, a marker of right ventricular dysfunction, has been shown in adult studies to correlate with pulmonary hypertension, a leading cause of death in SCD. VEGF, a marker of angiogenesis and endothelial cell activity, has correlated with pulmonary hypertension in some studies, but not in others.

Objectives: Our hypothesis is that both Pro-BNP and VEGF will be elevated in children with SCD, and that levels will correlate with conventional markers of severe disease.

Design/Method: 80 patients with SCD disease had Pro-BNP and VEGF levels collected in addition to a CBC, reticulocyte count, bilirubin and LDH during their regularly scheduled sickle cell clinic appointment. Patients with acute illness or pain crisis were excluded. The median age of patients was 8 years (range: 0.33–19 years). Pearson correlation coefficients were calculated between Pro-BNP, VEGF and continuous variables. The relationships between Pro-BNP, VEGF and genotype were evaluated by analysis of variance.

Results: There was little correlation between Pro-BNP and other known markers of severity of disease in SCD. The only exception to this was thrombocytosis. In contrast, elevated levels of VEGF correlated with most of the markers that were studied, including Hgb SS genotype, severe anemia, leukocytosis, thrombocytosis, a high reticulocyte count, elevated bilirubin and LDH, and hypoxemia.
OUTCOMES OF ADULTS WITH SICKLE CELL DISEASE

(POTTER 249)

NATIONWIDE UTILIZATION OF PACKED RED BLOOD CELL TRANSFUSIONS FOR THE TREATMENT OF UNCOMPROMICATED IRON DEFICIENCY ANEMIA

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Background: Uncomplicated Iron deficiency Anaemia (IDA) is treated with oral and parenteral iron. It is generally accepted that packed red cell transfusions be reserved for those with symptoms of congestive heart failure. Administration of PRBCs to patients who could have been safely treated with iron preparations adds cost to care and exposes them to avoidable risks of transfusion and could add to the cost of care.


Design/Method: National Inpatient Sample, a stratified probability sample of 20% of all US hospital discharges was used. Iron deficiency anemia was identified using ICD-9 code 280.0, 280.1 and 280.9 and ICD-9 code 99.04 was used for packed red blood cell transfusion.

Results:

- Utilization of PRBC for transfusion of IDA as primary diagnosis showed an increase from 38.2% to 49.5% from the year 2001 to 2007 (P < 0.05).
- PRBC is more likely to be administered for the primary diagnosis of Iron Deficiency Anaemia in children (43.7%) in comparison to adults (28.7%) (P < 0.05).
- The treatment of iron deficiency anemia with PRBCs is associated with significant additional economic burden with mean charges with and without PRBCs being 18,059 $ vs 14,225 $ (P < 0.05).
- Children with Iron Deficiency Anemia secondary to inadequate dietary iron intake are more likely to receive PRBC as compared to adults 59.0% vs 12.3% . Congestive heart failure was diagnosed in only 0.6% of patients who were transfused for a primary diagnosis of IDA.

Conclusion: Uncomplicated Iron deficiency anemia in children is frequently treated with packed red cell transfusion even in the absence of cardiac failure or associated co-morbidities. These data have significant implications on patient safety and cost of health care. These data underscore the need for prospective studies aimed towards optimizing the treatment of this condition.

LIVER BIOPSY IN CHRONICALLY TRANSFUSED CHILDREN WITH SICKLE CELL ANEMIA AND STROKE

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Background: Chronic transfusion therapy for secondary stroke prevention in children with sickle cell anemia (SCA) is often associated with transfusion iron overload. Liver biopsy provides quantitative liver iron content (LIC) plus histological information regarding fibrosis and inflammation, but carries risks for procedural complications.

Objectives: To determine the safety and pathology results of surveillance liver biopsy in the setting of a multicenter clinical trial.

Design/Method: Screening liver biopsies were performed on subjects enrolled in Stroke With Transfusions Changing To Hydroxyurea (SWITCH, NCT00122980) to document LIC >5 mg Fe/gm dry weight liver. Clinical sites had discretion for local biopsy procedures, to obtain three 1-cm biopsies for LIC, histology, and special studies.

Results: 150 liver biopsies were performed across 25 sites. Almost all were percutaneous biopsies, either transcapsular (81%) or coxial (63), with 3 surgical and 2 transjugular procedures. 14g needles were most frequent (68), followed by 16g (51) with a 14-21G range. Half were performed by interventional radiologists (76), followed by gastroenterologists (52), surgeons (16), and pediatric radiologists (5). Imaging guidance, usually ultrasonography, was used in 77%, while 63% had general anesthesia; in 35% the percutaneous tract was embolized. Only 47% received overnight hydration before biopsy. Mean hemoglobin both pre- biopsy and post- biopsy was 10.7 ± 1.3 gm/dl. Ten subjects (6.7%) had biopsy-related complications, none fatal or requiring transfusion. Complications included Grade 2 pain (6) or fever (3); two Grade 3 toxicities included pain from three transcapsular biopsies using a 14G needle and pulmonary aspiration despite CT guidance and general anesthesia. LIC averaged 15.5 mg Fe/gm dry weight liver (range 0.8 - 413), including 13 subjects with low LIC. Histology was usually adequate (124) or marginal (20), with only 6 (4%) deemed inadequate for interpretation. Almost all biopsies had grade 3-4 iron deposition, while 87% revealed lobular inflammation and 60% had some degree of fibrosis.

Conclusion: Liver biopsy in chronically transfused children with SCA is feasible and safe in a multicenter trial setting with local site procedural discretion. LIC is frequently highly elevated with substantial hepatic hemosiderosis, inflammation, and fibrosis.

LIVER BIOPSY IN CHRONICALLY TRANSFUSED CHILDREN WITH SICKLE CELL ANEMIA AND STROKE

Funded by NHLBI (U01-HL078787) with deferasirox study treatment supplied by Novartis, Inc.

POTTER 251

A PROLONGED QTc IS NOT ASSOCIATED WITH INCREASED MORTALITY IN SICKLE CELL DISEASE

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Background: An increased prevalence of prolonged corrected QT intervals (QTc) in patients with sickle cell disease (SCD) has been reported. The finding of a prolonged QTc has been correlated with an increased risk of sudden death in otherwise healthy individuals. No data exist defining the mortality risk of a prolonged QTc in SCD.

Objectives: Define the mortality risk of a prolonged QTc in patients with SCD.

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Design/Method: A retrospective review of patients with SCD (SS) at our institution in ambulatory and inpatient settings between January 2001 and June 2007. All ECGs obtained in patients with SCD during the study period were analyzed. Patients were defined as having prolonged QTc if the QTc measured > 460 msec on one or more ECGs. A patient was defined as having borderline QTc if the patient did not meet the definition of prolonged QTc but had one or more ECGs with a measurement of 440-459 msec. All ECGs were read by two pediatric electrophysiologists and Bazett’s formula was used to calculate QTc. The mean of the QTc measurements was used for analysis. An intra-class correlation (ICC) coefficient was used to calculate level of agreement.

Results: Of the 597 patients in our cohort, 201 (33.7%) had one or more ECGs during the study period. Age at ECG was 12.9 ± 5.5 years. Six patients were lost to follow-up. Of the remaining 195, 31 patients (15.9%) had prolonged QTc, and 60 (30.8%) had borderline QTc. There was agreement between the electrophysiologists in their measurements of the QTc (ICC coefficient = 0.756, p-value < 0.001). The mortality rate in the composite of the borderline and prolonged QTc groups was 4/91 (4.4%). The mortality rate in the normal QTc group was 3/104 (2.9%). This difference was not significant (p = 0.71).

Conclusion: Though the prevalence of borderline and prolonged QTc is increased in SCD, it is not associated with increased mortality in this cohort. Further studies are necessary to determine whether the increased prevalence of prolonged QTc is related to complications and therapies specific to SCD or to an increased prevalence of primary repolarization abnormalities.

(PAPER 252) PARENT-REPORTED QUALITY OF LIFE IN A COHORT OF PATIENTS WITH THALASSEMIA

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Background: Quality of life (QOL) has not been well studied in thalassemia, despite its importance as an outcome of treatment.

Objectives: To evaluate parent-reported QOL in a cohort of pediatric thalassemia patients.

Design/Method: Parents of 99 patients enrolled in the Thalassemia Longitudinal Cohort (TLC) study completed the Children’s Health Questionnaire (CHQ) PF28. The CHQ is a self-administered survey with 12 scales describing parental assessment of a child’s physical wellbeing, mental health, and family context. We expand on our previous findings that children with thalassemia have lower parental reported QOL than US norms, with associations by age, gender and chelator, and use multivariate modeling to adjust for significant factors.

Results: Patients had a mean age of 9.7 years (range 5.0-13.8), 48% male, 56% Asian, 91% chronically transfused. There were no significant differences in QOL by race, country or complications. Parents reported better QOL for patients on deferasirox versus deferoxamine in physical functioning, family activities, and physical summary score. Adherence (difficulties remembering or taking oral/SC chelator, and preparing/sticking pump for SC infusions) was negatively associated with parent-reported QOL for bodily pain, parental impact-emotional, mental health, behavior, family cohesion, and psychosocial summary score. In a multivariate model including age, chelator, gender and race, age was negatively associated with QOL for role-physical, bodily pain, mental health, and physical summary score; parents reported higher QOL for girls for role-emotional/behavior, mental health and psychosocial summary score; oral chelator use was associated with higher QOL in physical functioning, bodily pain, family activities and physical summary score, but lower QOL for behavior.

Conclusion: We show that the child’s age, gender and chelator choice are independently associated with parental report of QOL, and found associations between adherence and QOL. These data validate the observational evidence that a child with thalassemia has a significant impact on the entire family.

(PAPER 253) PRESCRIPTION CONTRACEPTION USE IN ADOLESCENTS AND YOUNG ADULTS WITH SICKLE CELL DISEASE: ANALYSIS OF MICHIGAN MEDICAID CLAIMS

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Background: Patients with sickle cell disease (SCD) have an increased risk of venous thromboembolic events (VTE). Because estrogen-containing contraception may further increase this risk, the World Health Organization classifies SCD as Category 2 for combined hormonal contraception (CHC) and Category 1 (no restrictions) for progestin-only contraception (POC). However, depot medroxyprogesterone (DMPA), commonly used in adolescents, is associated with bone mineral density loss, a complication already seen in SCD.

Objectives: The objectives of this study were to describe contraceptive agents most frequently prescribed to females with SCD and to determine the prevalence of VTE, pregnancy, and osteopenia.

Design/Method: We identified all women with SCD, aged 14–24 years, enrolled in the Michigan Medicaid Program between 1/1/2000 and 12/31/2003. The population was divided into two age cohorts (ages 14–18 and 19–24) due to differing Medicaid eligibility requirements. Using inpatient, outpatient, and pharmacy files, data was extracted regarding VTE, osteopenia, and pregnancy.

Results: In total, 31 patients in the study were 408 females with SCD. Of these, 47% were 14–18 years of age. Only 55 patients had pharmacy claims for hormonal contraception; 32 DMPA (58%), 20 CHC (36%), and 3 intrauterine devices (5%). Sixteen patients had VTE (4 associated with hormonal contraception) and 8 had osteopenia. In the 14–18 years cohort, 49 pregnancies were identified; 35 subjects had one pregnancy, 13 had two, and 1 had three during the four year study period. Only 12 of these 49 adolescents (25%) had post-partum pharmacy claims for contraception. In the younger age cohort, both pharmacy claims for contraception and number of pregnancies were higher than national averages for African American adolescents.

Conclusion: Our study suggests significant gaps exist in family planning care for young women with SCD. Female Medicaid enrollees may be more likely to seek family planning from Title X clinics such as Planned Parenthood, resulting in an absence of data for these services. However, the high number of pregnancies in our adolescent cohort suggests that this limitation does not fully explain our findings of low rates of contraception use, and that under-utilization of contraception is a true problem in the SCD population.

(PAPER 254) INCREASED PREVALENCE OF FALSE POSITIVE NEWBORN HEMOGLOBINOPATHY SCREENING IN PREMATURE INFANTS

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Background: Universal newborn screening for hemoglobinopathies detects children with sickle cell and other hemoglobinopathies at birth. Positive screening for disease requires confirmatory testing. In our experience premature infants appeared more likely to have false positive results at screening than term infants.

Objectives: The objective of this study was to investigate the accuracy of the screening in premature neonates compared to term neonates in identifying the presence of hemoglobinopathies.

Design/Method: We analyzed de-identified data from the Florida Newborn Hemoglobinopathy Screening Program for the years 2002-2007 regarding results obtained by isoelectric focusing for positive hemoglobinopathy screening for disease (not trait), confirmatory results, and gestational age. Relative risks for false positives for preterm and full term newborns were calculated by chi-square.

Results: 2300 neonates were suspected to have hemoglobinopathy based on the newborn screening (1/576 neonates born in Florida). The most common abnormal pattern in term infants suggesting disease at screening was FS, followed by FCA, FSC, FSA, and FC. In preterm infants (gestational age 22–36 weeks), the most common abnormal pattern was FS, followed by FSC, FSA, FCA, and FC. FSC was confirmed in 96% of the cases in both preterm and term infants. Overall, 90% of the children who screened positive for FCA and 65% of infants identified with FSC were later confirmed with trait. Compared to term newborns, preterm newborns were more likely to have a false positive result for FS or FC at screening which was later not confirmed (p < 0.00001). 23 % of pretermers with FS and 59% of pretermers with FC were diagnosed as traits by confirmatory testing, compared to only 2% and 6% respectively for term infants.

Conclusion: As compared to term newborns, more preterm newborns with trait were misidentified as having sickle cell anemia or hemoglobin C. We speculate that abnormal hemoglobin may precede the development of hemoglobin A during fetal life.

(PAPER 255) ABDOMINAL ULTRASONOGRAPHY FINDINGS IN CHRONICALLY TRANSFUSED CHILDREN WITH SICKLE CELL ANEMIA

Beth McCarville, Banu Aygun, Lee Hilliard, Margaret Lee, William Owen, Zora Rogers, Sharada Sarnaik, Paul Scott, Karen Kalinyak, Nancy Yovetch, Russell Ware, The Investigators Of The Stroke With Transfusions Changing To Hydroxyurea (SWiTCH)

St Jude Children’s Research Hospital, Memphis, Tennessee, United States
Background: Children with sickle cell anemia (SCA) have chronic hemolytic anemia and a high incidence of pigmented (bilirubin) gallstone formation. Splenomegaly and splenic sequestration can also occur, and surgical splenectomy may be required. The effects of chronic transfusion therapy on the development of gallstones and splenomegaly in children with SCA are unknown.

Objectives: To determine the prevalence of gallstone formation and splenomegaly in a large population of children with SCA and stroke, who receive chronic transfusion therapy for secondary stroke prophylaxis.

Design/Method: Stroke With Transfusions Changing to Hydroxyurea (SWITCH, NCT0012980) is a multicenter trial of children with SCA and clinical stroke, who have developed transfusional iron overload. Abdominal ultrasonography was performed at study entry according to a specified protocol, and then interpreted by a single central reader. Spleen volume was estimated using the maximum length, anterior-posterior, and transverse dimensions as follows: $L \times W \times H \times 0.523$ to adjust for the ellipsoidal shape of the spleen.

Results: A total of 148 abdominal sonograms were performed at 25 clinical sites, with 146 adequate for central interpretation. The average subject age was 12.9 years (range 5.0–19.0 years) with average transfusion duration of 7.1 ± 3.8 years (range 1.3–15.5 years). The gallbladder was absent in 36 (25%) due to prior surgery; another 46 (32%) had gallstones that were typically numerous and mobile, while gallbladder sludge without gallstones was observed in 11 (16%) additional children. The spleen was absent in 32 (22%) subjects due to presumed splenectomy; many of the remaining sonograms showed splenomegaly with an average volume of 283 ± 202 mL (range 10–1206 mL normal 100–150 mL), and 21 sonograms (19%) revealed substantial splenomegaly with >500 mL estimated volume. Four subjects had accessory spleens identified.

Conclusion: These baseline SWITCH data indicate that children with SCA and previous stroke have a high prevalence of cholelithiasis or gallbladder sludge, despite receiving chronic blood transfusions. Transfusion therapy may be associated with a high prevalence of previous splenectomy or current splenomegaly, suggesting that transfusions may delay autotransploncytosis.

Funded by NHLBI (U01-HL078787) with deferasirox study treatment supplied by Novartis, Inc.

(POSTER 256) NATIONAL ESTIMATES OF HEALTHCARE UTILIZATION FOR SICKLE CELL VASOCCLUSIVE CRISSES: SHORTER HOSPITAL STAY AND LOWER COSTS FOR PEDIATRIC PATIENTS

Lakshmanan Krishnamurti, Tanuja Gandhi, Ruchika Goel, Prabhu Viswanathan, Saksham Chandra

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Background: Vaso-Oclusive Crisis (VOC) is the commonest cause for hospitalization due to Sickle Cell Disease (SCD). While in the US, >70,000 people have SCD and 2 million carry the sickle cell trait, limited data exists on the National comorbidities and other factors affecting sickle related VOC.

Objectives: To determine the proportion of sickle cell disease (SCD) patients being seen in the ED who require hospitalization and factors contributing to the above are poorly understood.

Objectives: We present here analysis of such potential factors from the largest nationally representative ED visit data till date.

Design/Method: Nationwide Emergency Department Sample (NEDS), the largest all-payer ED database which approximates a 20-percent stratified sample of U.S. hospital-based ED’s for year 2006 was used for the analysis.

Results: A total of 166,043 ED visits happened with SCD as primary diagnosis. Of these visits, 68,420 (41.2%) resulted in admission to the hospital.

Conclusion: There was a statistically significant difference ($p < 0.001$) in the proportion of ER visits resulting in hospitalization for the following factors upon multivariable analysis:

1. Children and the elderly were more likely to be admitted to the hospital: The proportion of admissions was 50.7% in 1–17 yrs, 38.7% in 18–44 yrs, 44.0% in 45–64 yrs and 69% in the 65-84 yrs age group.

2. Metropolitan vs. non-metropolitan hospitals: Patients were more likely to be admitted to the hospital if they were seen in an ED in a metropolitan area (42.1% vs. 33.0%).

3. Insurance status: Patients with insurance coverage of any type were overall more likely to be admitted to hospital as compared with those without insurance (42.3% vs. 30.5%). Among the insured patients, the percentage admission was as follows: Medicare 39.9%, Medicaid 42.0% and privately insured 45.4%.

4. Residence in a low income area predicted a higher likelihood of subsequent hospitalization (40.0% vs. 41.8%).

5. Teaching hospitals were more likely to admit patients from the ED as compared to non-teaching hospitals (44.5% vs. 36.0%).

In a multivariate model, age emerges as a significant predictor of hospital admission even after 1) adjusting for presence of acute chest syndrome, fever and asthma and 2) excluding infants. OR = 1.38 (1.14-1.67).

Conclusion: High quality ED care for SCD is believed to result in a decrease of avoidable hospitalizations. However, the multiplicity of factors associated with subsequent hospitalization suggest the need for caution in using the proportion of ED visits resulting in hospitalizations as a surrogate marker of quality of care.

(POSTER 258) PREVALENCE RATE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS COLONIZATION IN HOSPITALIZED PEDIATRIC SICKLE CELL DISEASE PATIENTS

Beng Fuh, William Dalzell, Andrea Whitefield, Cynthia Brown, Teresa Fisher, Cathleen Cook

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Background: The background prevalence rate of Methicillin-resistant Staphylococcus aureus (MRSA) colonization has been published in regards to the general public and selected admissions. Risk factors for invasive MRSA include anatomic use, family contact with MRSA infections, medical contacts, and patient colonization with MRSA. For sickle cell disease patients, there is added risk of MRSA colonization due to the baseline immunocompromised state. However, MRSA colonization prevalence rates for hospitalized pediatric sickle Cell disease patients have not been reported.

Objectives: Determine the prevalence rate of MRSA colonization in Pediatric sickle cell disease patients.

Compare the prevalence rate of MRSA colonization in hospitalized pediatric sickle cell disease patients relative to the hospitalized general pediatric patients.

Design/Method: Community-based Teaching Hospital with a 30 bed Pediatric unit; retrospective review of charts from January 1, 2006 to December 31, 2009, non-duplicated general and sickle cell disease patients aged 0–18 years, PCR based MRSA screening results obtained within 24 hours of admission.

Results: For the three year period, there were 5661 children admitted to the General Pediatric Ward. Overall, 505 of 5661 (8.92%) children were nasally colonized with MRSA, of which 31 of 196 (15.8%) were sickle cell disease patients and 474 of 5465 (8.67%) were non sickle cell disease patients.

Conclusion: There is a high prevalence rate of MRSA colonization in hospitalized pediatric sickle cell disease patients. It is not clear what impact MRSA colonization has on invasive MRSA in pediatric sickle cell disease patients. Sickle cell disease patients have baseline immunocompromised state and can be presumed to be at
increased risk for invasive MRSA. How to intervene is controversial and not all facilities screen for MRSA, however many now have algorithms that dictate checking for colonization in patients with high risk for colonization, such as long-term care facility residents. Consideration of screening pediatric sickle cell disease patients should be considered as prevalence rates of colonization are higher than a general pediatric population and there is higher risk for invasive disease.

**(POSTER 259)**

PERCEPTIONS OF HOW RACE AFFECTS HEALTH CARE DELIVERY IN A SICKLE CELL PROGRAM

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Children’s Hospitals and Clinics of Minnesota, Minneapolis, Minnesota, United States

**Background:** There are approximately 90,000 Americans with sickle cell disease, with a prevalence of about 1 in 500 Blacks. Health care disparities based on race have been reported in the management of many diseases. Given the overwhelming majority of whites in the health care system, the effect of race on health care delivery to non-whites in Minnesota may be significant.

**Objectives:** Our goal was to evaluate perceptions of race and racism among staff and patients in the sickle cell program at Children’s Hospitals and Clinics of Minnesota, which serves over 90% of sickle cell patients statewide.

**Design/Method:** A confidential survey addressing issues of race and health care was given to all patients with sickle cell disease and their families upon arrival to clinic. The survey was made available online to all staff in the hematology/oncology program. We received completed surveys from 112 patients/families and from 135 staff. Both staff and families were predominately female (90.3% vs. 73.6%, p < 0.001). 92.6% of patients/families identified as black, while 94.1% of staff identified as white (p < 0.001).

**Results:** More patients/families felt that race affects the quality of health care for sickle cell patients (50% vs. 31.6%, p = 0.003). The majority of staff and patients/families agreed that racism is a problem in the United States, but not at our institution. More patients/families felt that all Americans have equal opportunities for success (64.1% vs. 44.1%, p < 0.001). More staff perceived racism as a problem nationally (83.9% vs. 72%, p = 0.02). Of respondents who felt there was racism at the hospital, more staff perceived this to be an issue (23.7% vs. 9%, p = 0.002). More staff perceived unequal treatment of patients at our institution, especially in the inpatient setting (20.9% vs. 10.9%, p = 0.03).

**Conclusion:** Racial health care disparities continue to exist in our country. Patients and families perceived this to be true more so than staff. Staff perceived racism as a problem in our institution more than did our patients and families. Theories to explain our findings will be presented. We plan to pursue social justice training to address issues of power, oppression, white privilege, societal resources, and structural barriers at our institution.

**(POSTER 260)**

WIND SPEED AND NOT TEMPERATURE IS ASSOCIATED WITH PAINFUL EVENTS IN CHILDREN WITH SICKLE CELL DISEASE IN THE TWIN CITIES

Stephen Nelson, Arianna Lund

Children’s Hospitals and Clinics of Minnesota, Minneapolis, Minnesota, United States

**Background:** The most prevalent complication of sickle cell disease (SCD) is pain. There have been conflicting reports about the role climate plays in the incidence of painful events in sickle cell disease. Smith et al. recently reported an association between low temperature and pain in adults with SCD. Given our northernly latitude and inland location, the Twin Cities experience the coldest climate of any major metropolitan area in the United States.

**Objectives:** We report relationships between climate and painful events in children with SCD at Children’s Hospitals & Clinics of Minnesota.

**Design/Method:** Between January 1st, 2003–December 31st, 2008 at Children’s Hospitals and Clinics of Minnesota there were 184 sickle cell patients with 677 painful episodes. We gathered temperature and wind speed data during the same time period using the Minnesota Climatology Working Group.

**Results:** The number of monthly painful episodes ranged from 41 (August and December) to 79 (May). The average monthly temperature ranged from 17.6 °F in January to 76.0 °F in August. The average monthly wind speed ranged from 7.78 mph in August to 10.34 mph in April. The frequency of painful episodes did not correlate with the average monthly temperature (R = 0.29, p = 0.17). However, we found significant correlation between higher average monthly wind speed and the increased prevalence of painful episodes (R = 0.75, p = 0.002). The highest prevalence of pain was in the spring and the lowest was in the winter (n = 199 vs.146, p = 0.001). The highest mean wind speed was in the spring and lowest was in summer (10.05 vs. 8.35 mph, p = 0.03). Wind speed was also significantly higher in spring when compared to winter (10.05 vs. 8.9 mph, p = 0.03).

**Conclusion:** The climate shifts during the season changes in Minnesota can be striking. High wind speed was significantly associated with painful events in our study. We are educating patients and families about these climate-related associations with painful events. Particular attention to appropriate dress during the changes in season may help prevent painful episodes. Further study is needed to identify other potential factors associated with pain in order to direct future education and prevention strategies.

**(POSTER 261)**

DECREASED FREQUENCY OF VASO-OCCLOSIVE CRISES ASSOCIATED WITH INTRAVENOUS IMMUNE GLOBULIN IN A PATIENT WITH SICKLE CELL DISEASE

James Cooper, Sriya Gunawardena, Lakshmanan Krishnamurti

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**Background:** In 2004, Turhan et al reported that the administration of IVIG to mice with sickle cell disease appeared to show inhibition of red and white cell interactions and improved blood flow. Chang et al reported similar findings in a 2008 sickle mouse model. We describe the results of a case of myasthenia gravis occurring in a 15-year-old girl with sickle cell disease who required monthly intravenous immune globulin (IVIG).

**Objectives:** To investigate retrospectively if murine model observations of decreased complications following intravenous immune globulin were observed in a child with sickle cell disease.

**Design/Method:** To examine the effects of IVIG treatment, the electronic medical record was reviewed to collect data on the frequency of admissions, admitting diagnoses, and duration of hospital stays. Data was collected for the two-year period before and after the diagnosis of myasthenia gravis. The timing of IVIG doses was also examined.

**Results:** Prior to her diagnosis of myasthenia gravis, our patient was admitted 17 times in 2 years for painful crises. In the 2 years after her diagnosis, she was admitted only 11 times for pain. While the median duration of each pain crisis was unchanged (7 days for both), the median interval between admissions increased significantly (24.5 days before diagnosis vs. 31 days after). The decrease in admission frequency even more noticeable during the second 12 months following her diagnosis, after IVIG infusions were scheduled rather than given upon symptom development.

**Conclusion:** To date, there have been no reports of myasthenia gravis in a patient with sickle cell disease. While this combination led to several therapeutic challenges, the regular administration of IVIG appears to have ameliorated our patient’s sickle cell disease phenotype. Previous reports have suggesting that IVIG may be beneficial due to decreased cell adhesion and to reduced macrophage activation. While our experience is limited, the role of IVIG in limiting vaso-occlusive crises should be further investigated in translational and clinical trials.

**(POSTER 262)**

CONCOMITANT USE OF HYDROXYUREA AND DEFERASIROX IN CHILDREN WITH SICKLE CELL ANEMIA

Bанию Айгун, Jane Hankins, Winfred Wang, Eileen Hansbury, Lynn Wynn, Amy Kimble, Russell Ware

St. Jude Children’s Research Hospital, Memphis, Tennessee, United States

**Background:** Children with sickle cell anemia (SCA) and recurrent vaso-occlusive episodes treated with chronic erythrocyte transfusions can be switched to hydroxyurea to prevent further episodes. However transfusion-acquired iron-overflow remains a problem in these children, who can require chelation therapy for several years after stopping transfusions. To date, there have been no reports about the concomitant use of hydroxyurea and deferasirox, the only FDA-approved oral iron chelator in the US.

**Objectives:** To describe the tolerability, safety, and efficacy of the concomitant use of hydroxyurea and deferasirox in children with SCA who had been chronically transfused for an average of 37 ± 3 months.

**Design/Method:** IRB-approved retrospective chart review of patients treated concomitantly with hydroxyurea and deferasirox at a single institution.

**Results:** Seven children (5 males, 2 females) with HbSS and history of acute chest syndrome or stroke were concomitantly treated with hydroxyurea and deferasirox. Six children had hydroxyurea at a stable maximum tolerated dose (MTD) for an average of 28.5 ± 7.5 months prior to starting deferasirox; one child was already on deferasirox…
Results: Seven children with this syndrome have been studied. They were 4 males and 3 females. All of them presented quiet early in life with a mean age of presentation of 83 days, with severe pallor, splenomegaly and required PRBCs transfusion. None had painful episodes. They were all transfusion dependent thereafter. 2 of them, started to have frontal and parietal bossing at presentation. The mean Hb at presentation was 4.3 g/dL. The Mean Hb S level was 25.8 percent and Hb S Oman 11%. Rest of the Hemoglobin was F and A2. Their blood fill was remarkable with typical Napoleon hat.
OBJECTIVES: To evaluate the frequency of PH in a group of pediatric patients with SCD living at high altitude (2000 mts above level sea).

RESULTS: This series include fifteen patients with a median age of 10.7 years (range 1-18). Clinical data are in table 1. Four patients (26.7%) were found with PH. The hemolytic crises were 10 in the group with PH versus 6 in the group without PH (p=0.07) and the BNP value in patients with PH was 157 versus 99 in those without PH (p=0.74).

Conclusion: PH could be developed early in patients with SCD who lives at high altitude and hemolysis crises are relate to this complication. We suggest for patients with SCD who lives at high altitude to be follow for this complication earlier.

Tables/Charts:

<table>
<thead>
<tr>
<th>Table 1: Characteristics At Diagnosis of Patients With SCD</th>
<th>PH</th>
<th>No PH</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>10.7</td>
<td>10.2</td>
<td>.06</td>
</tr>
<tr>
<td>Gender (male)</td>
<td>7 (46.7)</td>
<td>5 (31.3)</td>
<td>1</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>8.8 (0.0-11.1)</td>
<td>9.1 (8.0-11.5)</td>
<td>.51</td>
</tr>
<tr>
<td>Sickle Cell Count (%)</td>
<td>62 (30-100)</td>
<td>65 (30-100)</td>
<td>.64</td>
</tr>
<tr>
<td>Basophil Count (%)</td>
<td>0.0 (0.0-2.0)</td>
<td>0.0 (0.0-2.0)</td>
<td>.88</td>
</tr>
<tr>
<td>Hemoglobinopathy</td>
<td>9 (56.3)</td>
<td>5 (31.3)</td>
<td>.07</td>
</tr>
<tr>
<td>Cardiac disease</td>
<td>4 (25.0)</td>
<td>2 (12.5)</td>
<td>1</td>
</tr>
<tr>
<td>Prolonged QT interval (msec)</td>
<td>125 (100-150)</td>
<td>120 (100-150)</td>
<td>.18</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>4 (25.0)</td>
<td>5 (31.3)</td>
<td>.70</td>
</tr>
<tr>
<td>ELF (mg/L)</td>
<td>113 (75-218)</td>
<td>119 (80-227)</td>
<td>.80</td>
</tr>
<tr>
<td>BNP (ng/mL)</td>
<td>99 (50-100)</td>
<td>157 (75-250)</td>
<td>.07</td>
</tr>
</tbody>
</table>

PH: Pulmonary Hypertension; SCD: Sickle Cell Disease; ELF: Estimation of liver fibrosis; BNP: Brain Natriuretic Peptide.
TRANSCRIPTOME IDENTIFIES MULTIPLE PROTEIN (POSTER 270)

the potential development microRNA based therapeutics for neuroblastoma and other microRNAs suggests a strong tumor suppressor effect in vitro and in vivo, supporting microRNAs suppressed by MYCN in neuroblastoma whose expression correlates with expression of these microRNAs in a large group of patient samples (98 primary tumor specimens) demonstrates positive correlations with survival, suggesting that the MYCN-suppressed microRNAs oppose the oncogenic effects of MYCN.

Conclusion: With an unbiased genomic approach (ChIP-seq) we identified a panel of microRNAs suppressed by MYCN in neuroblastoma whose expression correlates with survival in patient samples. Functional analysis of mir-591 and several other microRNAs suggests a strong tumor suppressor effect in vitro and in vivo, supporting the potential development microRNA based therapeutics for neuroblastoma and other MYC-driven cancers.

(PPOSTER 270) NEXT GENERATION SEQUENCING OF THE NEUROBLASTOMA TRANSCRIPTOME IDENTIFIES MULTIPLE PROTEIN DISRUPTING MUTATIONS

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Background: Neuroblastoma is a small round blue cell tumor of childhood. Fifty percent of patients present with high risk disease and despite aggressive multimodal therapy approximately 60% percent of these patients die from their disease. Currently, only a handful of molecular alterations are known to influence prognosis, but no clear mechanism of pathogenesis has been demonstrated.

Objectives: To increase our understanding of the biology of neuroblastoma while identifying new targets for therapy, laying the groundwork for personalized treatment of this complex cancer.

Design/Method: We sequenced the transcriptomes of 20 stage four tumors, including ten MYCN amplified and ten MYCN non-amplified samples, using massively parallel sequencing technology. In our analysis pipeline, 50 nucleotide filtered reads are aligned to the reference human genome (hg 18). Reads that align were analyzed for: 1) base coverage, 2) transcript expression levels, 3) calling SNVs and 4) determination of damaging SNVs by Sorting Intolerant From Tolerant (SIFT) analysis.

Results: Initial analysis of the first six samples, yielded an average of 86.6 million uniquely mapped reads per sample. On average we detected the expression of 6,000 genes to a depth of 10x. The RNA seq expression profile correlated well with expression array data from the same sample (r = 0.62), while the sequencing data identified an additional 3,000 genes, not detected by microarray. Using the SAMtool, an average of 1,255 nonsynonymous SNVs predicted per sample. Of these nonsynonymous SNVs, 69-160 per sample were predicted by the SIFT algorithm to be damaging. Interestingly, 10 different genes had damaging nonsynonymous SNVs in at least 20% of the samples.

Conclusion: Next generation sequencing of transcriptome is a powerful and more sensitive method than microarrays for expression profiling and allows for the identification of novel transcripts including non-coding RNAs. Here we report the most extensive profiling of the neuroblastoma transcriptome to date. We identified several hundred protein disrupting SNVs, and of these 10 were commonly altered. Ongoing analysis is underway to validate our results. The identification of recurrent genetic alterations in NB will assist in developing a better understanding of the mechanisms of pathogenesis of neuroblastoma and lead to new therapeutic targets.

Identification and Characterization of Cancer Initiating Cells in Osteosarcoma

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Background: There is an urgent need to identify novel approaches to therapy and prognosis stratification in osteosarcoma. Currently, there are no available molecular markers to identify patients likely to have poor response to chemotherapy. Recent studies are limited by the lack of tumor availability and cell lines with well-annotated clinical correlation. Previous attempts to identify gene expression changes correlated with chemotherapy response have been hampered by low sample number and stromal contamination of tumor. Recent data suggests that OS tumors may contain subpopulations of cells with increased cancer-initiating capacity. Further analysis of this “cancer stem cell” population may provide novel insight into the pathogenesis and chemoresistance of OS.

Objectives: Establish a panel of primary patient cell lines and xenografts for OS Identify cell surface markers that may enrich for OS cells with increased tumorigenic capacity. Identify gene expression profiles that correlate with poor chemoresponsiveness.

Design/Method: We have initiated a multi-institutional collaboration (Stanford, UCSF, and Seattle) to acquire fresh tumor samples from patients undergoing a diagnostic biopsy or post-chemotherapy resection for osteosarcoma. We have established a reproducible method to xenograft OS samples into immunocompromised mice. These xenografts, along with the corresponding primary tumor samples were then analyzed for a range of cell surface markers using flow cytometric analysis (FACS).

Results: 10 xenografts (6 from biopsy, 4 from resection) from primary tumor samples have been obtained. Using FACS analysis, we have identified six surface markers that are heterogeneously expressed in primary tumor samples and the corresponding xenografts. Two of these markers (CD146 and CD49f) can enrich for subpopulations of tumor cells with increased colony-forming ability. In addition, chemoresponsiveness of cell lines derived directly from patient samples has been analyzed and correlated with gene expression.

Conclusion: We have established a multi-institutional collaborative study for the biology of human osteosarcoma. A panel of patient-derived cell lines and xenografts with well-annotated clinical correlation regarding chemotherapy responsiveness and patient outcome has been established. Preliminary studies suggest that cell-surface marker analysis of primary patient samples can be used to enrich for tumor-initiating cells. Gene expression studies of these subpopulations in order to identify markers of OS pathogenesis and chemoresponsiveness are underway.

Early Detection Strategies Reduce Cancer Mortality in Germline TP53 Mutation Carriers in Li-Fraumeni Syndrome

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Background: Li-Fraumeni Syndrome (LFS) carries a staggering lifetime risk of developing cancer. However, to date, routine biochemical and radiographic surveillance has been discouraged due to the lack of evidence supporting its effectiveness.

Objectives: The purpose of this study was to determine the impact of a comprehensive clinical surveillance strategy on clinical outcome in TP53 mutation carriers.

Design/Method: A clinical surveillance protocol for LFS has been implemented at The Hospital for Sick Children in Toronto and has been adopted by several institutions in the United States. TP53 mutation analysis and complete family histories, including site and age at diagnosis of neoplasms, were prospectively collected for 8 LFS families who have members participating in this protocol.

Results: Among the eight families, 49 TP53 mutation carriers were identified. The surveillance protocol detected 9 tumors in 6 of 16 TP53 mutation carriers who were screened. All 16 are alive after a mean follow-up time of 38 months compared with 11/ 33 (33.3%) TP53 mutation carriers who did not undergo surveillance (p = 0.029 x 0.06). All 6 TP53 mutation carriers (100%) who developed cancers identified by the surveillance protocol are alive with no evidence of disease, compared with only 5/27 (18.5%) TP53 mutation carriers who developed cancers and who did not undergo surveillance (p = 0.417 x 0.10 = 4).

Conclusion: We demonstrate that a clinical surveillance protocol can detect asymptomatic neoplasms in carriers of a germline TP53 mutation, and can significantly reduce cancer mortality among these patients. This report therefore lends support for genetic screening and surveillance of patients suspected of having LFS.

Identification of Cancer Initiating Cells in Osteosarcoma

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Duke University, Durham, North Carolina, United States

Background: Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma of childhood and adolescence, thought to arise from mesenchymal cells developing...
along the skeletal muscle axis. Despite aggressive multi-modal therapies, patients with high risk disease have an overall survival rate of less than 20% at 5 years. New therapeutic targets are clearly needed. One possible source of targets is the Notch signaling pathway, which plays a key role in many cell fate decisions, including proliferation and apoptosis. In skeletal muscle, Notch signaling also functions to maintain a population of quiescent reserve myoblasts by preventing premature differentiation. Aneratt Notch signaling has been implicated in several pediatric cancers, however, there have been no studies evaluating the potential of Notch as a therapeutic target in RMS.

**Objectives:** Evaluate the expression of Notch pathway members in multiple RMS models and investigate Notch signaling as a therapeutic target in rhabdomyosarcoma.

**Design/Method:** Immunohits and RT-PCR were used to evaluate protein and mRNA expression of Notch signaling ligands, receptors, and target genes in human RMS cell lines. Genetic and pharmacologic inhibition of Notch signaling was accomplished using retroviral-mediated stable expression of Notch1 shRNA and a γ-secretase inhibitor, respectively. In vitro cell growth and in vivo tumor growth of RMS cells were assessed using MTT colorimetric assays and murine tumor xenograph models, respectively. These properties may explain the ineffectiveness of existing therapies targeting chemotherapy.

**Results:** Notch1 protein expression and mRNA expression of multiple Notch signaling pathway ligands, receptors, and target genes are increased in RMS cells when compared to primary human skeletal muscle myoblasts. Stable knockdown of Notch1 in RMS cells inhibits cell growth in vitro and tumor growth in vivo. Pharmacologic inhibition of Notch signaling using γ-secretase inhibitor decreases RMS cell growth in vitro; in vivo inhibition is underway.

**Conclusion:** The Notch signaling pathway is upregulated in RMS cell lines, and inhibition of this pathway using both genetic and pharmacologic approaches impedes RMS cell and tumor growth. Notch signaling may function by preventing differentiation of RMS tumor cells. This is the first study evaluating inhibition of Notch signaling as a therapeutic target in RMS.

**(POSTER 274)**

**TARGETING CANCER STEM CELLS IN OSTEOSARCOMA**

Nino Rainusso, Alexa Ghazi, Vita Salsman, Helen Heslop, Stephen Gottschalk, Jeffrey Rosen, Nabil Ahmed

**Background:** Cancer stem cells (CSC) have been shown to drive tumorigenesis in a number of malignancies and may play a similar role in osteosarcoma. Mounting evidence has demonstrated that this subpopulation of tumor cells is more resistant to chemotherapy. These properties may explain the ineffectiveness of existing therapies for recurrent and metastatic disease. Therefore, new strategies that target osteosarcoma CSC could improve current patient outcome.

**Objectives:** HER2 expression regulates CSC in breast cancer, and correlates with poor prognosis in osteosarcoma. We propose to target osteosarcoma CSC using T-cell receptors expressing a HER2-specific chimeric antigen receptor (CAR) as novel immunotherapeutic approach.

**Design/Method:** Established osteosarcoma cell lines were grown as spheres (sarcospheres) in low attachment plates with serum-free medium as a surrogate CSC model. We analyzed the expression of CD133, an established CSC marker, and HER2 by flow cytometry. We also tested the ability of HER2-specific T lymphocytes to affect the formation of secondary sarcospheres.

**Results:** We observed by FACS analysis that 3-5% of the total osteosarcoma cells were simultaneously CD133+ and HER2+ positive. Moreover, in all osteosarcoma cell lines analyzed, the CD133+ positive cell fraction expressed higher levels of HER2 in comparison to the CD133-negative cells. Green transduced osteosarcoma cell lines were plated in conjunction with T-lymphocytes under sphere assay conditions. We noted that the efficiency of secondary sarcosphere formation was significantly reduced only in the group exposed to HER2 specific T-cells. The number of secondary sarcospheres exposed to non-transduced T-cells was similar to the control group in which no T-cells were added. In addition, using osteosarcoma cell lines we have developed an orthotopic xenograft mouse model that now enables us to recover different tumor cell subpopulations for serial transplantation experiments. Using this model experiments are underway to test the in vivo effect of HER2 specific T-cells on osteosarcoma RMS and their ability to form bone tumors and pulmonary metastases.

**Conclusion:** Our results indicate that adoptive immunotherapy targeting HER2 may effectively eliminate the osteosarcoma CSC population in vitro, which hopefully will translate into better tumor control in vivo.

**(POSTER 275)**

**CCER2-TRANSduced CYTOTOXIC T Lymyocytes SHOW ENHANCED HOMING IN VIVO TOWARD CCL2-SECRETING NEOBLASTOMA: IMPLICATIONS FOR ENHANCED ADOPTIVE IMMUNOTHERAPY**

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**Background:** Adoptively transferred cytotoxic T lymphocytes (CTLs) expressing a chimeric antigen receptor (CAR) targeting the GD2 antigen have shown promise against neuroblastoma in human trials, but the strategy requires improvement. Preclinical studies showed that GD2-expressing CTLs did not home to neuroblastoma xenografts, suggesting that homing of infused CTLs to tumor sites may be suboptimal. A chemokine predominantly produced by neuroblastoma is CCL2 (MCP-1), but CTLs used in clinical trials to treat neuroblastoma do not express CCR2. The receptor for CCL2. This deficiency may contribute to suboptimal trafficking to tumor sites and reduced clinical efficacy.

**Objectives:** To determine if genetic modification of CTL with CCR2 could increase homing to neuroblastoma.

**Design/Method:** CTLs were transduced using retroviral vectors encoding CCR2b, EGFP/CAR, or both.

**Results:** CTLs showed minimal expression of CCR2. ELISA for CCL2 was performed on established neuroblastoma cell lines and on neuroblastoma cell lines derived from patients primary tumors. Neuroblastoma cell lines were heterogenous for secretion of CCL2, but notably, all patient-derived primary tumor cell lines secreted high levels of CCL2. To test CTL homing towards CCL2, CTLs were transduced with a retroviral vector encoding CCR2b and subsequently expressed high levels of CCR2. CCR2b-transduced CTLs showed enhanced chemotaxis towards soluble CCL2 and towards supernatants from CCL2-secreting cell lines compared to non-transduced CTLs. To study in vivo migration, we co-transduced CTLs with EGFP-luciferase and CCR2b. Bioluminescent imaging of mice following intravenous injection of CTLs into mice bearing CCL2-secreting tumors (SK-N-SH) showed preferential migration of only CCR2b-transduced CTLs toward the tumors when compared to non-transduced CTLs. To determine if this enhanced homing would improve efficacy in vivo, we co-transduced CTLs with CCR2b and a CAR directed against GD2. CTLs transduced with GD2-CAR with or without CCR2b were intravenously injected into mice bearing CCL2-secreting tumors that had also been modified to express EGFP/luciferase (SK-N-AS-GFP/+/). Mice receiving CTLs co-transduced with CCR2b and GD2-CAR showed less tumor growth than mice receiving CTLs transduced with GD2-CAR alone.

**Conclusion:** These results demonstrate the feasibility of improving the trafficking and, thus, the efficacy of adoptively transferred T cells toward CCL2 secreting neuroblastoma thereby suggesting a potential means to enhance future adoptive immunotherapies.

**(POSTER 276)**

**DERAILLED RECEPTOR TYROSINE KINASE ENDOCYTOSIS IN GliOBlastOMA: SORTING NEXIN 3 DISRUPTS EGFR AND MET TRAFFICKING PROMOTING CELL PROLIFERATION AND TUMORIGENICITY**

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**Background:** Amplification/mutation or rearrangement of receptors tyrosine kinase (RTK) occurs in a large proportion of adult glioblastomas (GBM) (~60%) and plays a major role in gliomagenesis. Unlike aGBM, whereby oncogenic RTK signaling drives tumorigenesis and Ras activation, ascribes in a subset of pediatric astrocytomas. However, oncogenic up-regulation and activation of RTKs in children is driven by unique mechanisms which involve dysregulated SNX3 expression/activation.

**Objectives:** Based on our preliminary data, we hypothesize that the paradigm established for aGBM, whereby oncogenic RTK signaling drives tumorigenesis and Ras activation, applies in a subset of pediatric astrocytomas. However, oncogenic up-regulation and activation of RTKs in children is driven by unique mechanisms which involve dysregulated SNX3 expression/activation.

**Design/Method:** We stably overexpressed cMyC-tagged SNX3 in pGBM (SF188 and SN210) and aGBM (U87) cell lines. In parallel, we knocked-down SNX3 expression in these cell lines. We investigated the effects of overexpression/silencing of SNX3 on EGFR and cMET levels and activation, cell signaling (Ras and Akt pathways) and cell proliferation (monolayer/soft agar assays, xenograft mouse models).

**Results:** cMyC-tagged SNX3 overexpression increased RTK levels and delayed their degradation following EGFr/HG stimulation compared to empty-vector transfectants. This led to increased and sustained activation of the Ras pathway in SNX3 transfectants and a drastic increased in cell proliferation in monolayer and soft agar assays. Importantly, it increased tumor formation and tumor size in a xenograft nude mouse model. Silencing SNX3 in SF188 led to the reverse in knocked-down cell lines.

**Conclusion:** RTKs are major oncogenes in GBM. Keys findings in this study are that multiple biological events can lead to the same phenotype. In this instance, perturbing
endosomal recycling in GbM may lead to increased RTK signaling and cell proliferation similar to oncogenic mutations/genetic amplification affecting these molecules in a GbM. Importantly, we also show that gliomagenesis in children is driven by unique mechanisms.

(PAPER 277) ANTI-ANGIOGENIC AND ANTI-NEOPLASTIC EFFECTS OF THE MULTIKINASE INHIBITOR SORAFENIB IN NEUROBLASTOMA

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Background: Less than 40% of high risk neuroblastoma (NB) patients are cured with current treatments. Previous studies indicate that anti-angiogenic agents may be effective against high risk NB. Sorafenib, a multikinase inhibitor that prevents autophosphorylation of VEGFR, PDGFR, c-Kit and RET, potentially inhibits angiogenesis. It also blocks MAPK pathway signaling, indicating that it might be anti-neoplastic.

Objectives: To examine sorafenib’s effects on tumor blood vessels and NB cells. Design/Method: Proliferation was evaluated by MTT assay and MAPK pathway signaling by western blot analysis. Propidium Iodide was utilized to examine cell cycle distribution. Sorafenib’s effects on tumor growth were evaluated in NB xenograft and orthotopic models. SMS-KCNR (human) NB cells were injected subcutaneously into flanks of nude mice. Once tumors were palpable, mice were treated i.p. daily for 5 days/week, with 30 mg/kg Sorafenib orally or vehicle control for 10 doses. After two weeks tumors were harvested for analysis. To highlight vascular endothelial cells CD-31 staining was used. Tumor xenografts and orthotopic tumors, SMS-KCNR cells were injected into nude mice adrenal glands. Mice were treated as above. To highlight vascular endothelial cells CD-31 staining was used. Naive xenograft proliferation was assessed using Ki67. Ki67 and CD 31 positive cells in control vs. treated tumors were compared.

Results: Dose-dependent decrease in proliferation was seen following sorafenib treatment and ERK phosphorylation was inhibited by 55.4% (p = 0.004). Greatest inhibition occurred within 4 hours of treatment. Sorafenib treatment yielded a statistically significant increase in G1/S arrest in two NB cell lines (p = 0.003 and 0.021). Significantly decreased tumor growth was seen in xenograft models following sorafenib treatment (p = 0.016). Decreasing trend in size was seen in orthotopic animal models. CD-31 immunohistochemical staining was significantly decreased in treated xenografts (p < 0.001). Ki-67 staining showed an increase of GO (non-proliferating) cells in orthotopic tumors (p = 0.014).

Conclusion: Sorafenib inhibits angiogenesis, NB cell proliferation, and tumor growth. The anti-proliferative effects in NB cells are likely due, at least in part, to disruption of signaling via MAPK pathway which causes G1/S cell cycle arrest. These initial results indicate that sorafenib may be an effective therapeutic for children with high risk NB.

(PAPER 278) DEVELOPMENT OF AN AUTOMATED QUANTITATIVE METHOD FOR SCORING METIOBENZYLUGUANIDINE (mIBG) SCANS IN PATIENTS WITH NEUROBLASTOMA

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Background: Radiolabeled metioobenzyluguanidine (mIBG), a norepinephrine analog, is concentrated in cells of the sympathetic nervous system and is utilized in both diagnostic imaging and treatment of neuroblastoma. mIBG scans are typically interpreted qualitatively or semi-quantitatively by radiologists, often with poor inter-reader reliability. We have developed an automated quantitative method for scoring mIBG scans to overcome this inter-reader variability.

Objectives: Treatment decisions for children with neuroblastoma are often made based on results of mIBG scanning, and current methods are semi-quantitative at best. We sought to develop a computerized, automated segmentation and scoring algorithm for mIBG scan analysis, thus enhancing therapeutic decision-making.

Design/Method: With IRB approval, data from 70 mIBG scans from 17 patients with neuroblastoma treated at the University of Chicago Medical Center were collected for evaluation, and of these, raw data needed for further analysis was available for 25 scans from 11 patients. Images were scored by two experienced radiologists, according to the currently accepted standard of assigning each of 9 body segments a segmentation score of 0-3, depending on uptake. An automated, computerized segmentation algorithm was developed to divide the scan image into 9 segments and assign an extension score by relative mIBG signal intensity when compared to physiologic mIBG uptake in the liver. While fully automated, the radiologist had the option of adjusting the computerized segmentation where appropriate.

Results: Of a possible 250 events (25 scans with 9 segments and 1 total score each), our algorithm agreed with one or both of the radiologists 84.4% (211/250) of the time. The agreement between both radiologists was 44.4% (111/250) and between all three 39.6% (99/250). For total score, there was one false positive and no false negative events.

Conclusion: We have shown that an automated, quantitative method for recognizing mIBG uptake in patients with neuroblastoma is feasible and has superior reliability when compared to that of two subjective experienced radiologists. Such a system will enhance patient care, allowing therapeutic decisions to be more confidently based on changes in serial mIBG scans. A broader, prospective assessment of this method is warranted and should be incorporated into a clinical trial.

(PAPER 279) NOVEL USE OF MOLECULAR INVERSION PROBES TO INTERROGATE FORMalin-Fixed Parrafin-EMBEDDED (FFPE) SAMPLES OF EWING’S SARCOMA

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Background: Ewing’s Sarcoma (ES) is the second most common malignant bone tumor in children and young adults. Despite aggressive treatment, approximately 70% of non-metastatic and 30% of metastatic patients survive. Only patient’s age, tumor size, and location are known to predict outcome. Risk stratification based on molecular alterations may offer additional stratification of likely treatment response. Molecular Inversion Probes (MIPs) analyze genome-wide target sequences at high resolution and can detect both gene copy number and allelic imbalance in clinical samples, and have been demonstrated to work on archived formalin-fixed paraffin-embedded (FFPE) samples as old as 20 years.

Objectives: To use MIPs to detect copy number alterations (CNAs) in archived FFPE samples in Ewing’s sarcoma.

Design/Method: We extracted DNA from paired Ewing’s Sarcoma tumor samples and normal bone marrow aspirate lots from 7 pediatric patients diagnosed from 1997-2004 at Primary Children’s Medical Center at University of Utah. The MIP assay was run using the customized 330K Cancer Panel (Affymetrix), Santa Clara, CA). Copy number was calculated by comparing Ewing’s sarcoma samples to pooled normal control signal intensity for each probe. CNA calls were based on 5 consecutive probes with >90% call rate, standard deviation < 20%, and copy number ≤ 1.7 or ≥ 2.3.

Results: The 7 patients were ages 18 months - 18 yo (median 13 yo), 1 female and 6 males, and 1 metastatic bone disease (who later died of disease). MIPs revealed remarkably high-quality CNA data for each FFPE sample, including known and novel loci. Recurrent CNAs included gain at 1q21.3 - q44, 5p11, 8q11.22-23, 6p21.3, 8q23.3, 9p21.1, 12q21.3-31, 13q31.1, 15q11.1 - q11.2, 20q13.2, and loss at 1p33, 11q14.1, and 18p11.32. Conclusion: We believe this is the first time that high-resolution, genome-wide CNA data from FFPE samples in Ewing’s sarcoma have been reported. This is important because now it is possible to investigate an unlimited number of archived FFPE Ewing’s sarcoma samples. We now will be able to correlate CNAs with clinical outcome to elucidate likely treatment targets as well as discover new molecular alterations in Ewing’s sarcoma that may play a role in pathogenesis.

(PAPER 280) NOVEL STAT 3 INHIBITORS FLLL32 AND LLL12 INHIBIT STAT3 PHOSPHORYLATION AND INDUCE APOPTOSIS IN HUMAN OSTEOSARCOMA CELLS

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Background: The activation of signal transducer and activator of transcription 3 (STAT3) has been implicated in the oncogenesis of cancer and is regarded as a novel target for cancer therapy. Activated STAT3 is frequently detected in most types of human cancer including osteosarcoma, where it plays important roles in growth survival, drug resistance and angiogenesis. Osteosarcoma is a soft tissue sarcoma of childhood and adulthood, with an overall prognostic outcome of 60-70% overall survival when treated conventionally. Novel STAT3 inhibitors FLLL32 and LLL12 have been shown to block STAT3 pathway. We hypothesize that treating osteosarcoma cells with these small molecule inhibitors will induce apoptosis in vitro and suppress tumor growth in vivo.
Objectives: To study the effect of LLL12 and LLL11 on Osteosarcoma cells in vitro and in vivo.

Design/Method: Osteosarcoma cell lines, U2OS, S1OS2 and SU8A were treated with several concentrations of LLL12 and LLL11 and cell viability was evaluated by MTT analysis. Western blots were used to examine phosphorylation of STAT3, cleavage of caspase-3 and other protein at 24 hours post treatment with DMSO and the above inhibitors. RT/PCR was used to examine the effect of STAT3 inhibitors on STAT3 downstream target genes. Apoptosis was evaluated via PI and FITC Annexin v staining of cancer cells and flow cytometry. Osteosarcoma cells were injected into NOD/SCID mice and the experiment is ongoing.

Results: LLL12 and LLL11 were found to inhibit STAT3 phosphorylation and induce caspase-3 cleavage, which indicated the induction of apoptosis in osteosarcoma cell lines. LLL12 and LLL11 were also found to inhibit the expression of STAT3 downstream genes, Cyclin D1, Bel-41, and survivin, and also induced apoptosis (Annexin V-FITC+/− PI/) via flow cytometry analysis.

Conclusion: LLL12 and LLL11 inhibit STAT3 phosphorylation and induce cell death in Osteosarcoma cells in vitro. These novel STAT3 inhibitors have the potential to become therapeutic agents in the treatment of Osteosarcoma.

Objectives: We hypothesized that hypoxia would increase cellular proliferation of Saos-2 cells (p<0.05) was demonstrated under hypoxic conditions for Saos-2 cells. There was no change noted in arginase I mRNA in hypoxic Saos-2 cells. Increased cell proliferation (p<0.05) was demonstrated under hypoxic conditions for Saos-2 cells. Use of the arginase inhibitor S-(2-boronoethy)-l-cysteine, at concentrations of 10μM to 100μM prevented the hypoxia induced proliferation in Saos-2 cells (p<0.05). Transfection of small interfering RNA (siRNA) targeting arginase II in Saos-2 cells prevented hypoxic induction of arginase II protein and completely prevented hypoxia induced cellular proliferation. Hypoxic arginase II siRNA treated cells are significantly different from both hypoxic control and hypoxic scramble treated Saos-2 cells (p<0.005).

Conclusion: As hypothesized, hypoxia induces proliferation of Saos-2 cells through arginase II induction. This was supported by demonstration of decreased cellular proliferation using pharmacologic arginase inhibitors and specific siRNA against arginase II. Further studies investigating upstream pathways and signaling molecules are warranted. We speculate that arginase II inhibitors may represent novel therapeutic targets in treatment of solid tumors.

Objectives: To study the population consists of infants (<1 year of age at diagnosis) with non-metastatic RMS who were treated on IRS-IV, the low-risk D9602, or the intermediate-risk D9803 from 1991 to 2005. Eligible patients had Stage 1-3, Clinical Group (CG) I-II RMS and were assigned to receive multimodal therapy with chemotherapy ± surgery and/or radiotherapy. Data regarding clinical features, treatment received, and outcome for infants were collected and analyzed.

Results: Seventy-six infants with non-metastatic RMS were treated on the three protocols (IRS-IV:N = 41; D9602:N = 20; D9803:N = 15) and comprised 4% of total patients enrolled. Median age was 7.4 months (range 0.1–12 months); 13 patients (17%) were <3 months and 4 (5%) were <1 month. Tumor histology included embryonal/botryoid (61%), alveolar (21%), and undifferentiated/ectomesenchymoma (18%). Fifty-seven percent of infants had CG III tumors. Parameningeal primary site was less common in this infant cohort (5%) than for all patients treated on IRS-IV (25%). The estimated 4-year DFS and 4-year OS for the entire cohort are 58.5 ± 5.8% and 76.2 ± 5.1%, respectively. Alveolar histology and CG III tumors were predictive of worse outcome. Among the 32 patients with treatment failure, 20 had local recurrence or progression, 6 developed distant metastases, 3 had local and metastatic progression, and 2 died from toxicity. The median time to treatment failure was 1.1 years from diagnosis (range 1 month to 6 years), and 15 infants experienced treatment failure within one year of diagnosis. Fifty-six percent of infants with CG III tumors had radiation therapy withheld or received a lower dose than recommended by protocol.

Conclusion: Infants with RMS appear to have worse outcomes than older patients, in part due to high rates of local failure. Concerns regarding morbidity in infants and reluctance to perform aggressive local control measures may lead to higher rates of local failure.

Objectives: To examine pulmonary nodules for expression of Fasp and FasL, and presence of dendritic cells following GM-CSF inhalation, to estimate the event-free survival (EFS) and S of patients with pulmonary recurrence of osteosarcoma following this therapy and to determine if the maximum dose utilized in the adult trial of inhaled GM-CSF for melanoma is tolerable in pediatric patients.

Design/Method: Forty-three eligible patients received inhaled GM-CSF at doses ranging from 240–1750 mcg twice daily on alternate weeks. Following two cycles, patients underwent thoracotomy to resect tumor and analyze pulmonary nodules for expression of Fasp/FasL, presence of dendritic cells for following GM-CSF inhalation, to estimate the event-free survival (EFS) and S of patients with pulmonary recurrence of osteosarcoma following this therapy and to determine if the maximum dose utilized in the adult trial of inhaled GM-CSF for melanoma is tolerable in pediatric patients.

Results: The mean scores for Fas and FasL, in nodules from patients with bilateral recurrence who underwent unilateral thoracotomy pretreatment (using a scoring system of 0–3) pre treatment were 1.3 and 0.88 respectively, compared to 0.78 and 0.62 in nodules resected following two cycles of therapy. Only 11 of 30 nodules post inhalation were positive for CD1a, 4 of 30 for S100 and 6 of 30 for clusterin. EFS and S at 3 years were 7.8% and 35.4%, respectively. Dose escalation to a dose of 1750 mcg BID was feasible.

Conclusion: Inhalation of GM-CSF at doses from 240-1750 mcg twice daily on alternate weeks was feasible but did not result in detectable immunostimulatory effect in pulmonary metastases, nor did it result in improved outcome post resection.

GM-CSF provided by Berlex. Study support: National Cancer Institute.

Objectives: We describe the characteristics, outcomes, and patterns of failure for infants less one year of age with rhabdomyosarcoma who were treated on Intergroup Rhabdomyosarcoma Study (IRS) protocols IRS-IV, D9602, and D9803.

Design/Method: The study population consists of infants (<1 year of age at diagnosis) with non-metastatic RMS who were treated on IRS-IV, the low-risk D9602, or the intermediate-risk D9803 from 1991 to 2005. Eligible patients had Stage 1-3, Clinical Group (CG) I-II RMS and were assigned to receive multimodal therapy with chemotherapy ± surgery and/or radiotherapy. Data regarding clinical features, treatment received, and outcome for infants were collected and analyzed.

Results: Seventy-six infants with non-metastatic RMS were treated on the three protocols (IRS-IV:N = 41; D9602:N = 20; D9803:N = 15) and comprised 4% of total patients enrolled. Median age was 7.4 months (range 0.1–12 months); 13 patients (17%) were <3 months and 4 (5%) were <1 month. Tumor histology included embryonal/botryoid (61%), alveolar (21%), and undifferentiated/ectomesenchymoma (18%). Fifty-seven percent of infants had CG III tumors. Parameningeal primary site was less common in this infant cohort (5%) than for all patients treated on IRS-IV (25%). The estimated 4-year DFS and 4-year OS for the entire cohort are 58.5 ± 5.8% and 76.2 ± 5.1%, respectively. Alveolar histology and CG III tumors were predictive of worse outcome. Among the 32 patients with treatment failure, 20 had local recurrence or progression, 6 developed distant metastases, 3 had local and metastatic progression, and 2 died from toxicity. The median time to treatment failure was 1.1 years from diagnosis (range 1 month to 6 years), and 15 infants experienced treatment failure within one year of diagnosis. Fifty-six percent of infants with CG III tumors had radiation therapy withheld or received a lower dose than recommended by protocol.

Conclusion: Infants with RMS appear to have worse outcomes than older patients, in part due to high rates of local failure. Concerns regarding morbidity in infants and reluctance to perform aggressive local control measures may lead to higher rates of local failure.
Background: High-grade gliomas are one of the most aggressive of all human cancers, are highly resistant to radiation- and chemo-therapy and have either local or distant recurrence after treatment with surgery and/or radiotherapy. MRK is a stress-regulated MAP kinase kinase kinase (MAPKKK) that is activated by ionizing radiation (IR) and is required for invasion stimulated by growth factors in different settings. To study in vitro invasion and migration we used transwell chambers with or without extracellular matrix (Matrigel), respectively. RNA interference was performed using transient transfection of at least two different siRNA oligonucleotides, to control for off-target effects. Viability of cells post-IR was studied using the MTS assay. Results: Knockdown of MRK mRNA by siRNA-mediated depletion in glioblastoma cells inhibits IR-induced invasion and migration. We also found that depletion of either p38 or Chk2 inhibits IR-induced invasion and migration. Moreover, the inhibitory effect of Chk2 depletion on cell migration was reversed by blocking cell cycle progression. Conclusion: Our observations establish a critical role for MRK in IR-induced glioblastoma invasion and validate MRK and MRK-controlled signaling elements as novel therapeutic targets in the treatment of high grade gliomas. Our data also indicate that Chk2 contributes to IR-induced invasion via control of the cell cycle.

(POSTER 285) A COMPARISON OF TWO TREATMENT REGIMENS FOR GROUP B INTRAOCULAR RETINOBLASTOMA

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Background: The optimal chemotherapy for eyes with group B intraocular retinoblastoma has not yet been defined.

Objectives: We report our experience with 2 cohorts of patients treated non randomly with local ophthalmic therapy and three courses of low dose carboplatin, etoposide and vincristine (CEV) versus higher dose CEV.

Design/Method: We performed a retrospective analysis of 15 patients (19 eyes) with group B retinoblastoma who were treated with three courses of carboplatin (18.5mg/Kg), etoposide (10mg/Kg) and vincristine (0.05mg/Kg) from 1995-98. Our second group included 10 patients (11 eyes) with group B retinoblastoma who were treated with 3 courses of higher doses of carboplatin (20mg/kg), etoposide (10mg/Kg) and vincristine (0.05mg/kg) from 2000-2005. Both groups of patients received local chemotherapy with laser and/or cryotherapy during and after the chemotherapy regimen. Failures were defined as eyes which required additional chemotherapy, enucleation or external beam irradiation.

Results: Both chemotherapy regimens were tolerated well with expected myelosuppression being the only toxicity observed. Group I: As of 1/2009, 10/19 eyes continue to have no active disease and have not required further therapy. For the remainder: One patient developed a pinal primitive neuroectodermal tumor three years after initial diagnosis and one month later developed an intracanal edge recurrence and vitreous seeding. This patient died shortly thereafter. The remaining eight patients had edge recurrences, which were not amenable to local therapy alone. Six patients (nine eyes) were treated with a higher dose carboplatin or cytotoxan with etoposide/ vincristine. All are disease free for a median of 72 months (range 48–156). Two patients had the affected eye enucleated. Overall, 16/20 eyes were salvaged with systemic chemotherapy and local therapy alone. Group II: As of 1/2009, 7/11 eyes continue to have no active disease and have required no further therapy. Four eyes had edge recurrences that were treated successfully with local ophthalmic therapy. All are disease free for a median of 38 months (range 22–61).

Conclusion: Our data suggest that courses of higher dose CEV appears more effective than 3 courses of lower dose CEV for patients with Group B intraocular retinoblastoma. Further study is warranted in larger numbers of patients.

(POSTER 286) THE USE OF ZOLEDRONIC ACID IN CHILDREN WITH SOLID TUMORS

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Background: Zoledronic acid is a third generation bisphosphonate utilized in adult patients with malignancy and metastatic bone disease. In this population, zoledronic acid has proven efficacy in improving bone pain, reducing skeletal complications, and improving quality of life. Additionally, zoledronic acid has demonstrated antineoplastic properties in vitro, and is being evaluated as a component of multigent antimet tumor therapy in both adults and children. Zoledronic acid is underutilized in pediatric oncology, and its use may benefit patients at risk for skeletal complications related to their malignant disease.

Objectives: To review our experience with zoledronic acid in pediatric patients with solid tumors that involve the skeletal system.

Design/Method: Pediatric patients with solid tumors involving the bone that received at least one dose of zoledronic acid between October 2005 and December 2008 were identified using pharmacy records. A retrospective review of patient charts was performed at a single institution.

Results: A total of 18 patients received one or more doses of zoledronic acid as part of their treatment. Diagnoses were: neuroblastoma(6), Ewing’s sarcoma(4), osteosarcoma(2), undifferentiated sarcoma(2), rhabdomyosarcoma(1), PNET(1), rhabdoid tumor of the kidney(1), and desmoplastic small round cell tumor(1). The average age of patients was 12.5 years (range 1.1–23.1 years). The most frequent reason for administration was pain control. Dosing was variable and ranged from 0.04 mg/kg to 0.19 mg/kg every four weeks, with 11 patients receiving the recommended adult dose of 4 mg. 10 patients received multiple doses (range 1–26 doses), with 7 patients receiving greater than 10 total doses. Asymptomatic hypercalcemia and hypophosphatemia were common and did not require intervention. Severe side effects associated with zoledronic acid use in adults were not seen, including osteonecrosis of the jaw and renal insufficiency.

Conclusion: Zoledronic acid is an option for pediatric patients with solid tumors that involve the bone to improve pain symptoms and reduce skeletal complications. Zoledronic acid is well tolerated with a favorable toxicity profile. Further evaluation in pediatric oncology is warranted. Based on adult guidelines, patients should have a dental evaluation prior to administration and calcium supplementation is recommended.

(POSTER 287) ARE THE JUVENILE PILOCYTIC ASTROCYTOMAS THE NAEVI OF THE BRAIN?

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Background: Low grade gliomas (LGG) including juvenile pilocytic astrocytomas (JPA) constitute a relatively frequent, heterogeneous and poorly understood subset of brain tumors in children. Using high resolution SNP-based microarrays, we identified and validated a recurrent tandem duplication on chromosome 7q34 that leads to a fusion between KIAA1549 and BRAF resulting in a constitutive active kinase involving part of the kinase domain of the BRAF oncogene in ~50%. LGG. This duplication occurs mainly in sporadic JPA ~63%, and rarely in other LGG. We also established that incidence of this duplication is site specific, as it occurs in the majority of cerebellar JPA (~80%).

Objectives: We hypothesize that the constitutive kinase activation in BRAF induces cell senescence in astrocytes, leading to a growth arrest of these premalignant lesions. We additionally believe that other mechanisms may lead to senescence in tumors not harbouring 7q34 duplication.

Design/Method: We transiently transfected wild-type and mutant (V600E) of BRAF plasmids into human TERT immortalized normal human astrocytes (NHA). We then investigated if this overexpression would trigger cell senescence in the cells. We tested senescence markers such as p16INK4a, p53 and b-galactosidase. We also performed immunohistochemistry using an antibody against p16INK4a and p53 in vivo on 46 JPAs.

Results: Stable transfections using wild type and the mutated form (V600E) of BRAF in NHA demonstrated a loss of senescence markers suggesting that high expression levels of BRAF might triggers cell growth instead of senescence. To get physiological expression levels of BRAF, we treated with BRAF and BRAFV600E were transiently transfection into NHA and showed an increased expression of p16 and p53. Immunohistochemical analysis using a p16 antibody showed a positive staining in 80% JPAs that harbour the duplication and in 70% JPAs that do not have the duplication, confirming the senescent nature of JPAs.

Conclusion: Our findings suggest that oncogenic induced senescence is promoted in JPAs as it is also present in the nevi of the skin, and this may act as a barrier to tumor progression. Ultimately, the BRAF duplication could be used as a biological marker between low grade and high grade tumors and also as a prognostic marker in a clinical setting.
CLINICAL FEATURES OF PLEOMORPHIC XANTHOCYCTOTOMA
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Background: Pleomorphic xanthoastrocytoma (PXA) is a rare variant of low-grade astrocytoma which occurs primarily in children and young adults.

Objectives: Its clinical features remain to be elucidated because < 400 cases have been reported to date, most as single cases or small series.

Design/Method: Patient’s data were collected through ICD-O code of 9424 in the National Cancer Database and the Ohio Cancer Incidence Surveillance System and analyzed using standard statistical methods.

Results: Between 1996 and 2006, 577 patients were reported nationally. There were 287 males and 290 females with median age of 25.2 years (range of 2–86). Sixty-five percent of patients were Caucasian, 11.3% African-American, 11.3% Hispanic, and 12.1% for others. The overall annual incidence was 0.21 per million with the highest annual incidence of 0.51 per million in subjects between 10 and 30 years of age, leading to an estimation of 70–75 patients diagnosed annually in the U.S. Approximately 98% of tumors were supratentorial, with 37% in the temporal lobe, 18% in the parietal and 17% in the frontal. Ninety-one percent of patients had surgical resection including 28.1% with gross total resection and 62.9% with subtotal resection. The cumulative survival rate at 5 years was calculated along with patient’s age, primary tumor sites, and surgical resection. The overall relative survival rate was 85.7%. However, younger patients had a better survival rate (72% for <30 years vs. 47% for >30 years, p < 0.01). Tumor location also affected the relative survival rate with 77% for those with parietal lobe tumors (p < 0.05), 56% for those with temporal lobe tumors, and 47% for those with frontal lobe tumors. The extent of surgical resection dramatically affected the survival. Patients had 85% survival rate (p < 0.01) when they had a gross total resection, 62% with subtotal resection and 31% with biopsy only.

Conclusion: Upon review this is the first report to describe the incidence of this rare CNS tumor. PXA’s prognosis is relatively favorable, but dependent upon patient’s age, tumor location, and the extent of surgical resection. These data warrant further study nationally to better characterize its biological features.

DIFFERENTIAL ACTIVATION OF THE RAS PATHWAY IN RHABDOMYOSARCOMA SUBTYPES
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Background: Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma of childhood and adolescence, and generally manifests as either the embryonal (eRMS) or alveolar (aRMS) histologic subtype. To better understand RMS, we have generated primary cell-based genetically defined models of both subtypes. Using this model of eRMS, the small GTPase Ras is necessary for recapitulating the eRMS subtype. Oncogenic Ras mutations in human eRMS samples, and germ-line Ras mutations in Costello syndrome (predisposed to eRMS) support these findings. No mutations in aRMS have been described for aRMS, yet we find that the PAX3-FOXO1 fusion gene, a signature genetic change in aRMS, can functionally replace Ras in tumor modeling. Since PAX3-FOXO1 is upstream of two receptor tyrosine kinases known to signal through RAS (cMET and FGFR4), we predict that Ras is involved in aRMS tumorigenesis.

Objectives: To determine the overall and activated Ras levels in aRMS compared to eRMS, and to begin to characterize upstream and downstream signaling events in the Ras pathway in these tumor types.

Design/Method: We examined three eRMS and three aRMS human cell lines for overall and activated Ras expression using GST-mediated Ras-GTP pulldowns and immunoblots. To probe the upstream signaling events that contribute to Ras activation, we stimulated aRMS cell lines with FGFR and c-MET ligands to examine their effect on Ras-GTP levels. In addition, the activation of downstream Ras effectors MAPK/ERK, RAL-GEF, and PI3K/AKT was examined to further characterize the function of Ras signaling in RMS tumorigenesis.

Results: Overall and activated Ras levels are significantly higher in eRMS cell lines, consistent with our prior eRMS modeling. In aRMS cells, basal activation of Ras was minimal, and only inducible in response to FGFR but not c-MET axis signaling. Moreover, the PI3K/AKT pathway is preferentially upregulated in aRMS but not eRMS cell lines.

Conclusion: This study provides insight into Ras activation in the two most common human RMS variants and will help develop targeted use of Ras pathway inhibition in the treatment of this pediatric cancer. Ongoing efforts are focused on confirming these findings in human RMS tumor tissue samples.

REDEFINING THE INCIDENCE AND OUTCOMES OF CNS ATYPICAL TERATOID Rhabdoid TUMOURS AT BC CHILDREN’S HOSPITAL
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Background: Atypical Teratoid Rhabdoid Tumour (ATRT) of the central nervous system (CNS) is a rare and highly malignant embryonal neoplasm. A specific antibody test to detect loss or mutation of the INI-1 tumour suppressor gene (hSNF5/SMARCB1) has been used at BC Children’s Hospital (BCCH) since 2007. Prior to the availability of this test, ATRT’s could be indistinguishable from other tumours by routine pathology, and therefore may have been under-diagnosed.

Objectives: We applied the INI-1 test retrospectively to determine how many CNS embryonal tumours over the prior two decades would be classified as an ATRT. Identifying any ‘cryptic’ ATRT’s allows us to better describe the clinical course for this unique tumour, which historically has been reported to have a dismal prognosis.

Design/Method: With local ethics approval, paraffin-embedded tissue was retrieved from storage for all BCCH patients from 1986–2006 who were diagnosed with CNS embryonal tumour. Slides were prepared and re-stained with the anti-BAF47/INI-1 antibody, and histologic features were reviewed by a neuropathologist.

Results: Ninety-four patient samples were available, and INI-1 staining showed loss of retention in 12 samples (including 2 previously reported as ATRT without INI-1 testing). Median age at presentation was 1.3 years (0.3–9 years), and there was a 9.3 female: male ratio. One patient was treated with radiation, and 2 had metastatic disease. Median survival was only 14 months, but there were three long term survivors (4, 6, 8 years) without relapse. Revealing these ‘cryptic’ ATRT’s changes the incidence of ATRT from 4% (<1/25) to 36% (<25) of all <3 year old patients with embryonal tumours between 1966–2006.

Conclusion: Previously there had been no reported survivors of ATRT at BCCH; now 3/12 (25%) have >3 year survival rate. Two were young patients treated with intensive chemotherapy and no radiation, showing promise for this strategy. A better understanding of how many children have actually had ATRT’s and their corresponding survival rates is critical for designing more specific treatments in the future. Capturing ATRT as a separate entity inherently allows a more accurate reporting of survival rates for other CNS embryonal tumours.

MyCN IS ASSOCIATED WITH LOSS OF mir-206 IN A GENETICALLY DEFINED MODEL OF ALVEOLAR RHABDOMYOSARCOMA
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Background: Rhabdomyosarcoma (RMS) is the most common soft tissue sarcoma of childhood and adolescence. Despite new therapies and improved supportive care, survival for high risk RMS, especially the alveolar histologic variant (aRMS), is less than 50% at 5 years. To understand its molecular pathogenesis, we previously created a genetically defined model of aRMS in which primary human skeletal muscle myoblasts are transformed step-wise into tumorigenic cells that mimic aRMS. Using this model, we noted that the addition of MycN was critical to confer resistance to skeletal muscle differentiation. However, the specific role of MycN in preventing differentiation is not known. mir-206 is a skeletal muscle-specific microRNA that promotes myogenic differentiation in mammalian cells. In rhabdomyosarcoma, mir-206 expression is suppressed, preventing differentiation. Therefore, we investigated if MycN expression correlated with mir-206 loss in our genetic model of RMS.

Objectives: To measure miR-206 expression in normal human log phase myoblasts, differentiated myotubes, and in our genetically defined model of aRMS. If indeed mir-206 is downregulated, to gain insight into the mechanism of this loss.

Design/Method: The genetically defined model of aRMS has been previously validated at both the biochemical and biological levels (Namn, Cancer Research, 2008). miR-206 levels were measured by real time PCR, controlled to RNU44 levels. Primary human skeletal muscle myoblasts or aRMS model cells were subject to a standard skeletal muscle differentiation assay. Standard statistics were used to quantify data significance.

Results: miR-206 levels appropriately increase as primary human skeletal muscle cells are induced to differentiate. mir-206 levels persist in our genetically defined model of aRMS until the expression of MycN, concomitant with the loss of ability to
differentiate. Additionally, MyoD levels are also stable until MycN is introduced, suggesting a mechanistic link between MycN, MyoD, and mir-206 expression. **Conclusion:** mir-206 is associated with the differentiated phenotype and is lost in human RMS. Using our genetically defined model of aRMS, we find that mir-206 is lost concomitantly with the expression of MycN, and in association with the loss of MyoD. Future work will examine the role of MycN in MyoD-regulation of mir-206 expression.

**(POSTER 292)**

**RETINOIC ACID PROMOTES PROTEOSOMAL DEGRADATION OF REST AND NEURONAL DIFFERENTIATION IN NEUROBLASTOMA CELLS**

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**Background:** Neuroblastomas are tumors that arise from neural crest progenitors. The RE-1 Silencing Transcription factor (REST) is a negative regulator of neuronal differentiation and is expressed at high levels in several neuroblastoma cell lines. REST expression is associated with an undifferentiated phenotype in these cells. Retinoic Acid (RA) is used in the treatment of neuroblastoma in an attempt to differentiate these cells. The underlying mechanism of differentiation is not completely understood. **Objectives:** To study if RA modulates REST expression and promotes differentiation in neuroblastoma cells.

**Design/Method:** SK-N-SH (RA sensitive) and SK-N-AS (RA resistant) cells where treated with RA (10μM) and changes in the expression of REST and its target genes were measured by Quantitative Real-Time RT-PCR, Western Blotting (WB), and Immunofluorescence assays (IFA). Tumor cells were treated with RA +/- MG132 and REST levels studied by Western blotting.

**Results:** The levels of REST protein were higher in SK-N-AS than in SK-N-SH cells at baseline. Treatment with RA induced differentiation in SK-N-SH cells but not in SK-N-AS cells. IFA and WB showed treatment with RA causes a decrease in REST and an increase in the expression of REST target genes: Type-III beta tubulin (IFAT), and Synapsin I (WB), resulting in neurite formation in SK-N-SH cells but not in SK-N-AS cells. Interestingly, REST transcript levels were unaffected in both cell types upon RA treatment. Given that RA targets proteins for proteasomal degradation we asked if it has an effect in REST protein degradation. Co-incubation of SK-N-SH cells with RA in the presence or absence of the proteasomal inhibitor MG132 showed an increase in REST levels in the RA-treated cells.

**Conclusion:** RA causes a down-regulation of REST and neuronal differentiation in SK-N-SH cells but not in SK-N-AS cells. This decline in REST protein expression in SK-N-SH cells occurs through increased proteasomal degradation of REST. Thus, RA may induce differentiation by promoting REST degradation in neuroblastoma cells. The evaluation of REST expression in patient samples may help distinguish who would benefit more from the use of RA in neuroblastoma treatment.

**(POSTER 293)**

**ROLE OF DLX2 HOMEOBOX GENES IN RETINOBLASTOMA DIFFERENTIATION**

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**Background:** Retinoblastoma (RB) is the most common ocular malignancy of childhood and presents either as non-hereditary, unilateral or hereditary, often bilateral lesions. The oncogenic mutation is a two-hit mechanism, with one mutation being the bi-allelic inactivation of the Rb1 gene usually through loss-of-heterozygosity. The retinal cell-of-origin, in which these two mutations arise, is not confirmed. The Dlx homeobox genes are necessary to control differentiation in the retina. Unlike Rb1 heterozygote mice, Rb1/p107 compound mutants develop retinoblastoma. The developmental regulation of p107 is unknown. **Objectives:** We hypothesized that: (1) DLX2 is expressed in RB but not in ocular melanoma, a tumor of neural crest origin; (2) DLX2 co-expression with retinal cell-specific markers will help to identify the RB cell-of-origin; and (3) DLX2 regulates p107 during retinal development.

**Design/Method:** Immunohistochemistry and immunofluorescence studies were performed on wild-type and mutant mouse and human cryosections or FFPE tumor sections. Pax6 (G), A (H), Prox1 (H, A cells), and Syntaxin (A cells) were used as markers expressed in ganglion (G), horizontal (H) and/or amacrine (A) cells. We used chromatin immunoprecipitation (ChIP) combined with CpG island microarrays (chip) of embryonic mouse retina to identify candidate DLX2 transcriptional targets. **Results:** RB neoplastic cells express DLX2, Pax6, Prox1 and Syntaxin. However, ocular melanoma cells do not express DLX2. DLX2 is expressed in the outer nuclear layer in the adult human retina containing the cell bodies of photoreceptors, contrasting with the adult mouse retina, where DLX2 is localized to the inner nuclear (A, H) and ganglion cell (G and displaced A cells) layers. Using ChIP-chip, we identified regions of the p107 promoter bound to the DLX2 protein. **Conclusion:** Current developmental models for RB favor a transitional cell as the cell of origin in mouse and humans. Our results support this model, since DLX2 expression signifies cells committed to a neuronal lineage. Our discovery of DLX2 expression in the cell bodies of the human ONL underlies the importance of studying human tissues in addition to mouse models in order to understand human disease. Transcriptional regulation of the Rb family member p107 by DLX2 may be a mechanism whereby this homeobox gene promotes retinal differentiation.

**(POSTER 294)**

**HISTONE DEACETYLYASE INHIBITION AS THERAPY FOR ATYPICAL TERATOID/RHABDOID TUMOR: PRELIMINARY IN VITRO ANALYSIS**

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**Background:** Histone deacetylase inhibitors (HDIs) have emerged as promising therapies for multiple malignancies. HDIs maintain DNA acetylation, resulting in an open chromatin configuration. Genes involved in processes such as tumor suppression, cell cycle inhibition, apoptosis induction, and differentiation are often de-repressed. Atypical teratoid/rhabdoid tumor (ATRT) is a malignant central nervous system tumor seen mostly in young children. Historically, the median survival has been extremely poor; therefore, ATRT is a malignancy that requires evaluation of potential new therapies. **Objectives:** To evaluate the ability of HDIs to alter the in vitro proliferative capacity of ATRT cell lines and to analyze differential gene expression after HDI treatment.

**Design/Method:** BT-12 and BT-16 ATRT cell lines were treated for 72 hours with various concentrations of the HDIs trichostatin A (Tsa), suberoylanilide hydroxamic acid (SAHA), or MS-275. MTT assay was performed to determine relative cell number, and data was analyzed to determine IC50 values. Cells were again treated with serial dilutions of MS-275 from IC25 downward, collected, and re-seeded for clonogenic assay. Total RNA for both cell lines was collected after 24 hours of treatment with MS-275 and was subsequently loaded onto an Affymetrix gene chip. Results of the gene evaluation.

**Results:** Treatment with all three HDIs resulted in decreased relative cell numbers. IC50 values were as follows (BT-12 and BT-16, respectively): Tsa 253nM, 207nM; SAHA 3.8μM, 0.82μM; MS-275 275μM, 1.6μM. Clonogenic assay revealed decreased colony formation. BT-12: 60 to 18 colonies; BT-16: 93 to 51 (mean, control vs. IC25). Gene array analysis revealed differential up-regulation (minimum 1.75-fold change) of genes functionally grouped as immune response, cell adhesion, cell differentiation, and apoptosis. Down-regulation was most notable in gene clusters associated with cell cycle progression and DNA damage response repair.

**Conclusion:** HDIs demonstrate antiproliferative effects in vitro against the ATRT cell lines BT-12 and BT-16. Given the changes noted on gene expression analysis affecting processes such as cell cycle, apoptosis, and DNA repair, HDIs may prove useful in potentiating other chemotherapeutics or radiation and warrant further evaluation.

**(POSTER 295)**

**SUB-CELLULAR LOCALIZATION OF Y-BOX PROTEIN 1 REGULATES PROLIFERATION, INVASION, AND INCREASED MESENCHYMAL PHENOTYPE IN ASTROCYTOMAS**

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**Background:** Y-Box Protein 1 (YBX1) is a DNA/RNA-binding protein implicated in cancer progression. YBX1 is mandated for embryonic development and acts as a transcriptional/translational regulator in the cytoplasm. Akt-dependent phosphorylation of S102YBX1 induces nuclear translocation and potentially oncogenicity. **Objectives:** We previously established elevated YBX1 levels in Pediatric Glioblastoma Multiforme (GBM), an aggressive high-grade brain tumor, possibly driving oncogenesis in this cancer. We investigated herein the effects of stable knock-down or ectopic
Neuroblastoma, the most common extracranial tumor of childhood, is
Background:

DIFFERENTIATED CELLS CONSISTENT WITH THE CANCER STEM

POSTER 297) NEUROBLASTOMA TUMORSPHERES ARE ENRICHED FOR LESS
DIFFERENTIATED CELLS CONSISTENT WITH THE CANCER STEM
CELL HYPOTHESIS

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Background: Neuroblastoma, the most common extracranial tumor of childhood, is an excellent candidate for a cancer stem cell disease. First, there is significant dichotomy in clinical outcomes, with infants younger than 18 months having a good prognosis despite metastases and older patients with disseminated disease faring poorly even with aggressive therapy. Second, historical observations show that those patients whose tumor cells established cell lines in culture have a poor prognosis. Finally, tumors composed of multiple cell phenotypes share a single karyotype, suggesting that they arose from a single multipotent cell.

Objectives: We sought to phenotypically and molecularly characterize tumospheres, thought to be enriched for so-called cancer stem cells, from neuroblastoma cell lines.

Design/Method: We maintained neuroblastoma cell lines under standard conditions (“bulk”), in stem cell media to derive tumospheres (“spheres”), or passaged with aggressive washing to remove floating and loosely adherent cells to enrich adherence (“adherent”). We compared these subcultures for sphere-forming ability, expression of classic stem cell surface markers by flow cytometry, and differentiation status by RT-PCR. We also tested them for chemosensitivity. Finally, we evaluated tumorigenesis and cell homing to metastatic sites following injection into athymic nude mice.

Results: Spheres showed a remarkably increased sphere-forming ability in comparison to adherent cells, with bulk culture cells showing an intermediate sphere-forming capacity. Spheres showed differential responses were noted between the cell populations in their expression of classic stem cell markers, though spheres appeared to be the least differentiated of the cultures. In vivo tumorigenic and biodistribution studies are ongoing.

Conclusion: Neuroblastoma tumospheres exhibit a distinct phenotype from more differentiated cell lines in culture and may represent enrichment of tumor-initiating or cancer stem-like cells. Therapeutic targeting of such cells is likely to be a critical aspect of improving patient outcomes.

(POSTER 299) DRIVEN TO DEATH: FARNESYL TRANSFERASE INHIBITION CAUSES
A COUNTERINTUITIVE INCREASE IN RAS ACTIVITY, INCLUDING ERK AND P38 PATHWAY ACTIVATION, WHILE CAUSING CELL DEATH FOR SENSITIVE OSTEOSARCOMA LINES

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Background: Chemotherapy-related improvements in outcome have been negligible for osteosarcoma for 25 years, fueling interest in novel therapeutics. Highly specific kinase inhibitors provide little benefit, suggesting that inhibition of fundamental signaling processes, or of multiple targets, may be needed. One possible target is the prenylation pathway that anchors small molecule GTPases such as Ras to the plasma membrane. Inhibitors of farnesylation (FTI), a type of prenylation, have shown promise for some cancers, especially those lacking oncogenic Ras mutations. Given the apparent importance of several receptor tyrosine kinases upstream of Ras for osteosarcoma, we thought it possible that FTIs would impact osteosarcoma cell growth.

Objectives: Since osteosarcoma does not develop oncogenic Ras, we wished to know what impact FTI would have on osteosarcoma biology, both for cell survival and growth as well as for intracellular signaling.

Design/Method: Osteosarcoma lines OS187, COL and Saws-2 and 4 other cell lines were cultured in 1 nM to 1 micromolar tipifarnib, a small molecule FTI. Cell proliferation, cycle and viability were assessed by direct cell counting and PI analysis of cell cycle (flow cytometry). Ras localization was assessed by immunofluorescence. Invasion was assessed with matrigel. Western blot measured protein expression, and Ras activity was measured by binding to bead-conjugated Raf. No direct effects were noted between the cell populations in their expression of classic stem cell markers, though spheres appeared to be the least differentiated of the cultures. In vivo tumorigenic and biodistribution studies are ongoing.

Conclusion: Neuroblastoma tumospheres exhibit a distinct phenotype from more differentiated cell lines in culture and may represent enrichment of tumor-initiating or cancer stem-like cells. Therapeutic targeting of such cells is likely to be a critical aspect of improving patient outcomes.

Results: Tipifarnib decreased cell viability, colony formation, and tumor volume both in vitro and in vivo. Ras localization was altered in all cell lines, with increased Ras being detected in the cytoplasm and cytosol and decreased in the plasma membrane. Inhibition of Ras activity occurred in all cell lines, with increased activation of p38 and ERK1/2. Inhibition of Ras activity and MAPK signaling in sensitive osteosarcoma lines, causing cell death and growth arrest. Resistant lines still showed reduced invasion with FTI. If a marker can be identified for responsive tumors, some osteosarcoma patients may benefit from FTI.
Background: Chemotherapy is widely accepted as first-line therapy for pediatric low-grade gliomas (PLGG). However, treatment modalities for further progression are still controversial. Specifically, the role for repeated chemotherapy remains unclear.

Objectives: The aim of the study was to determine treatment outcome for PLAG treated by chemotherapy at recurrence.

Results: From 1985 to 2009, 118 patients received chemotherapy as first-line treatment for PLGG, 38 received chemotherapy as second line, with 13 of these patients treated again at further recurrence. The most common protocol at relapse was vincristine (60%) flowed by TPCV (thioguanine/ procarbazine/ lomustine/ vincristine) and vincristine/carboplatin, each used in 18% of the patients. Second-line chemotherapy was well tolerated and toxicity was comparable to first-line treatment. Strikingly, at a median follow-up time of 7.49 years, 5 and 10 years progression-free survival was 9% and 39%, respectively. Those with Grade 0 ototoxicity were more likely to have poor necrosis of tumor with an odds ratio of 3.75 and a 95% confidence interval of 1.07–13.17 (p =0.034). Final results will be presented at the meeting.

Conclusion: This large population based study demonstrates feasibility and favorable tumor control with repeated chemotherapy for recurrent PLGG. In contrast to other tumors, the benign nature of PLAG justifies consideration of less toxic therapeutic modalities at recurrence. High morbidity rate after long-term follow-up warrants further investigation.

(PAPER 300)

OTOTOXICITY AS A PREDICTOR OF OUTCOME IN PATIENTS WITH OSTEOSARCOMA TREATED WITH CISPLATIN

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Background: Currently almost all Osteosarcoma patients are treated with a chemotherapy protocol that includes Cisplatin. This platinum agent is known to cause irreversible ototoxicity in up to 60% of patients. Consequently, the use of Cisplatin in osteosarcoma patients and the degree of tumor necrosis is being evaluated as a predictor of outcome in vivo responses to chemotherapy, as all subjects have 12 weeks of chemotherapy prior to surgery. It has been well documented that the degree of tumor necrosis at surgery is correlated with patient outcome (inferior outcome if less than 90% necrosis).

Objectives: The objective of this study is to identify any correlation between cisplatin ototoxicity in osteosarcoma patients and the degree of tumor necrosis. We hypothesize that subjects with ototoxicity after 12 week neo-adjuvant therapy are more likely to have a poor histological response (defined as under 90% necrosis) when compared to those with hearing loss.

Design/Method: All individuals with osteosarcoma treated at a single institution between 1990 and 2009 were retrospecively analyzed. All patients who received cisplatin and had complete data available (pathology at week 12, baseline and week 12 audiograms) were included. The cohort was separated into those with any degree of hearing loss (Grades 1 to 4 hearing loss as classified by CTCAEv3) and those with no hearing loss (Grade 0). The outcome measure was favorable tumor response (>90% necrosis) or poor response (< 90% necrosis).

Results: 67 subjects were identified and complete results are currently available for 44 subjects. Hearing loss (grade 1-4) was seen in 22 subjects (50%). Of the 22 subjects with no hearing loss, favorable necrosis was seen in only 7 (31.8%). Of the 22 subjects with grade 1-4 hearing loss, favorable necrosis was seen in 14 (63.4%). These preliminary results show that those with Grade 0 ototoxicity are more likely to have poor necrosis of tumor with an odds ratio of 3.75 and a 95% confidence interval of 1.07–13.17 (p =0.034). Final results will be presented at the meeting.

Conclusion: These preliminary results suggest that those with no ototoxicity are at increased risk for poor tumor response to neo-adjuvant chemotherapy. This raises the possible role of ototoxicity as a predictor of outcome in Osteosarcoma.

(PAPER 301)

THE PATTERN OF THE IMMUNE RESPONSE DIFFERS IN LOW- RISK VS. HIGH- RISK NEUROBLASTOMA

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Background: It has been suggested that failure of the host immune system in regression of neuroblastoma is in part due to lack of HLA expression and defects in antigen presentation machinery of the tumors. Accordingly, innate immune responses are thought to play an important role against neuroblastoma. Since innate immune responses are more active in younger children than adaptive immune responses and patients with low-risk neuroblastoma are usually younger than 18mths, we sought to determine which components of the immune system would be active in low-risk vs. high-risk neuroblastoma.

Objectives: To identify if patients with low-risk and high-risk neuroblastoma exhibit distinct immune function gene signatures and to determine if innate immune responses correlate with low-risk disease and adaptive immune responses correlate with high-risk disease.

Design/Method: Microarray analysis was performed on primary tumor lesions of neuroblastoma, focusing on tumor antigens and immune function genes. Real time PCR was used to determine relative quantity of the expression of selected genes. Flow cytometry analysis of peripheral blood to explore the status of lymphocytes and myeloid-derived suppressor cells (MDSC), and multiplex cytokine array of the sera for the detection of key cytokines associated with innate or adaptive immune responses were also performed.

Results: Microarray analysis showed differential clustering of immune function genes in high-risk vs. low-risk patients. The real time PCR revealed that low-risk patients had significantly higher expression of the tumor suppression gene TUSC3, whereas MDSC and IL-18 receptor1 were elevated in high-risk patients. Flow cytometry showed that low-risk patients also had a higher level of MDSCs and lower expression of NKGD2 as well as CXCR4 in their circulating lymphocytes. The multiplex cytokine array analysis of the sera showed higher levels of cytokines IL-1, IL-10 and MCP-1 in patients with low-risk neuroblastoma.

Conclusion: These data suggest favorable prognostic value of the signatures of immune function genes i.e. an innate immune response evidence, evidenced by increased IL-1, MCP-1, IL-18 receptor1, as well as a diminished adaptive immune response, evidenced by increased MDSC, USP12, IL-10 and decreased NKGD2 and CXCR4. Therefore, enhancing the innate immune response may result in improved outcome in patients with high-risk neuroblastoma.

(PAPER 302)

LAGTIME TO ONCOLOGIC DIAGNOSES: A COMPARISON BETWEEN MILITARY AND NON-MILITARY HEALTHCARE SYSTEMS

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Background: Surveillance, Epidemiology, and End Results (SEER) program data from 1992–2002 shows oncologic patients aged 15–19 years did not experience similar reduction in mortality as their younger counterparts. 2000–2004 data reveals adolescents and young adult (AYA) patients aged 15–29 years accounted for a disproportionate 75% of the total number of new oncology cases in patients aged less than 30. In 2007, Martin, et al. showed that AYA patients with private insurance had reduced lagtimes to diagnosis than those with public insurance. In the Military Healthcare system, access to care is not a variable in the lagtime equation as all beneficiaries have universal access.

Objectives: To compare the performance of the National Capital Area (NCA) oncology system with previously published private and public lagtime data.

Design/Method: Charts were reviewed from patients diagnosed at ages 15–29 years from 1 JAN 2000 to 31 DEC 2008 with sarcoma, non-Hodgkin’s lymphoma, and leukemia. Lag times were the difference between the first date of a related symptom to diagnosis. Lag times were compared to original data from Martin, et al using the Mann-Whitney test.

Results: Ninety-three charts revealed sixty evaluable lag times. Mean lag times for NCA diagnoses were compared to M.D. Anderson Cancer Center (MDACC) private and public insurance lagtimes. p values from NCA versus MDACC private insurance were: sarcomas (p =0.11), non-Hodgkin’s (p =0.26), and Leukemia (p =0.12). p values from NCA versus MDACC public insurance were: sarcomas (p =0.004), non-Hodgkin’s (p =0.029), and Leukemia (p =0.037).

Conclusion: There was no significant difference in the lagtimes to diagnosis between the military healthcare system and private insurance at MDACC. Lagtimes for both systems were significantly shorter that those with public insurance. As an example of universal healthcare, the military healthcare system is an important model of uninterrupted access to care; which, as these data show, is a significant factor in decreasing lagtimes to oncologic diagnoses. Further research to evaluate other possible factors that can lead to delays in diagnosis in the AYA population such as
feeling of immortality, poor health education, and avoidance should take into account this important variable (Martin, Oncologist, 2007).

**Background:** Carcinoid tumors (CTs) are rare in the pediatric population and generally found incidentally on histopathological examination. Cure is usually achieved with surgical excision. Second primary malignancies (SPM) of the gastrointestinal tract after CTs have been reported in 13 to 33% of affected adults. The rate of SPM appears highest after small bowel or appendiceal CTs and usually present within 7 years from diagnosis.

**Objectives:** To investigate whether children and adolescents with primary CTs of the appendix develop recurrence or SPM during routine long term follow up.

**Design/Method:** After approval by the Institutional Review Board, a retrospective review of medical records was conducted in children and adolescents with histological diagnosis of CTs of the appendix diagnosed at Nationwide Children’s Hospital since 1945.

**Results:** Twenty four patients presenting at a mean age of 13 years were identified. This series represented 0.43% of all malignancies and 0.25% of all appendectomies. Twenty one patients (88%) presented with clinical appendicitis and were incidentally found to have CTs of the appendix upon histopathological examination. Three patients underwent an incidental appendectomy at the time of a planned abdominal surgery. Four patients that presented with clinical appendicitis had CTs with invasive features, including invasion of the mesoappendix, vascular invasion, and one tumor > 2 cm. Three patients underwent hemicolectomy and one an Ileocelectomy due to invasive features. None of the 24 patients had symptoms of carcinoid syndrome or metastatic disease. All patients had localized disease were successfully treated by complete surgical excision and were free of disease 6 years from diagnosis. None of our patients including those with invasive features developed SPM of the gastrointestinal tract.

**Conclusion:** Carcinoid tumors are rare in pediatric populations and are usually an incidental finding. The overall prognosis for CTs in children and adolescents is excellent with long term survival after complete surgical resection. However, the possibility of recurrence and the possibly increased risk for a gastrointestinal SPM, such as adenocarcinoma of the colon, raises the importance of close follow-up and consideration of earlier colonoscopy in adults who as children or adolescents had CTs of the appendix.

**Design/Method:** A retrospective chart review was performed on all children from the Children’s Hospital of Eastern Ontario, Ottawa, Ontario, Canada.

**Objectives:** To determine types of gonadal tumors seen in the PPB FTS.

**Background:** Pleuropulmonary blastoma (PPB) is a rare embryonal tumor of early childhood. PPB is the sentinel disease in a distinctive family tumor syndrome (PPB FTS) recently found to be related to mutations in DICER1. Ovarian sex cord stromal tumors (OSCST), including Sertoli-Leydig cell tumors (SLCT), are also rare, and currently of unclear etiology.

**Objectives:** To determine types of gonadal tumors seen in the PPB FTS.

**Conclusion:** Carcinoid tumors are rare in pediatric populations and are usually an incidental finding. The overall prognosis for CTs in children and adolescents is excellent with long term survival after complete surgical resection. However, the possibility of recurrence and the possibly increased risk for a gastrointestinal SPM, such as adenocarcinoma of the colon, raises the importance of close follow-up and consideration of earlier colonoscopy in adults who as children or adolescents had CTs of the appendix.
sentinel node biopsy, of which 8 were positive--these 8 patients subsequently underwent lymph node dissection. Six patients received interferon, 4 radiation therapy, 2 GM-CSF, 2 interleukin and 2 adjuvant chemotherapy with Temozolomide. Of the entire cohort, 2 patients never achieved remission and 3 patients recurred. Two of the patients with recurrence are currently alive--1 patient was treated with radiation and interferon and has no evidence of disease 9 months after relapse; the second patient is currently receiving high dose interleukin. Median duration of follow up is 33 months (range: 5–59 months). Estimated overall survival at 4 yrs was 86% and estimated event free survival was 77%. Only the stage of melanoma at diagnosis was a statistically significant predictor of outcome (p = 0.02).

Conclusion: Similar to adult studies, outcome in our cohort correlated with clinical stage at time of diagnosis. This data emphasizes that a high index of suspicion is needed while evaluation of melanocytic lesions in children and adolescents--further reduction in mortality may be achieved by early detection.

(POSTER 307)
GENDER IS A PREDICTOR OF CISPLATIN OTOTOXICITY
Allison Yancey, Michael Harris, Akinbode Egbealan, Jaimie Gilbert, David Pisoni, Jamie Renbarger
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Background: Cisplatin is an effective agent against a variety of pediatric solid tumors. One of its main side effects is irreversible, bilateral, sensorineural hearing damage that is extremely variable from patient to patient.

Objectives: The aim of this study was to evaluate the incidence and possible predictors of cisplatin-related ototoxicity.

Design/Method: We performed a retrospective chart review of 128 pediatric patients who had completed cisplatin therapy for osteosarcoma, neuroblastoma, hepatoblastoma, or germ cell tumor. Patients were diagnosed at Riley Hospital for Children between January 1995 and June 2008, were less than 18 years old at diagnosis, and had normal hearing prior to therapy. Audiograms obtained at baseline, and on therapy, and off therapy were scored by using the Brock scale, a validated grading system for cisplatin-related hearing loss. Cumulative dose, dose adjustments, concurrent ototoxic medications, and disease status were also captured as part of the retrospective analysis.

Results: Forty-two percent of the patients studied experienced hearing loss of any grade and 28% had moderate to severe ototoxicity (Brock grade ≥ 2). Gender was a predictor of cisplatin-related ototoxicity; males were at significantly greater risk (four times more likely) for developing hearing loss than females (Odds ratio 4.812). While age and gender were independent predictors of cisplatin-related ototoxicity, the severity of ototoxicity may be inversely related to age, with very young patients exhibiting higher grades of hearing loss following cisplatin therapy.

Conclusion: Gender and cumulative dose are important predictors of cisplatin ototoxicity. The severity of ototoxicity may be inversely related to age, with very young patients exhibiting higher grades of hearing loss following cisplatin therapy.

(POSTER 308)
INCIDENTAL BRAIN LESIONS IN CHILDREN: TO TREAT OR NOT TO TREAT?
Amy-Lee Bredlau, David Korones
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Background: Many children are found incidentally on imaging of the brain to have masses that may not be related to the initial reason brain images were obtained. Because there are few data on the outcome of such children, we reviewed the course of a group of children followed at our center with brain lesions found incidentally on neuro-imaging.

Objectives: To determine the outcome of children who had incidentally found brain lesions on neuro-imaging.

Design/Method: A database of all children with brain tumors followed at the University of Rochester Medical Center from 2000-9 was reviewed, and data were extracted on original presentation, MRIs, intervention, progression, and follow-up of all patients with brain lesions found incidentally while being evaluated for unrelated symptoms or for trauma.

Results: There were 21 patients with incidental brain lesions. Their median age was 12 years (range 1-18); 11 (55%) were female. Nine patients (45%) had no symptoms and 12 (57%) had symptoms not felt to be related to their lesion. Twelve of the patients (57%) had lesions < 2cm, and the remaining 9 (43%) had lesions 2-5cm in size. Nine of the 19 (47%) lesions seen on MRI enhanced. The lesions were found in the cerebellum (n = 6, 29%), midline (n = 3, 14%), brainstem (n = 2, 10%), and cerebrum (n = 10, 48%). Six patients (29%) had surgery at presentation. Four of these six patients were treated with radiation for low grade glioma (3) and ependymoma (1). The remaining 2 patients had low grade glioma. Of the remaining 15 patients, 4 had progression of disease on serial MRI scans: three underwent surgery with or without radiation (2 with low grade glioma and 1 with a mature teratoma vs. dermoid cyst) and the fourth patient was monitored and remained stable after the initial progression stabilized. The progression free and overall survival of all patients were 81% and 100%, respectively, with a median follow-up of 20 months (range 1-104 months).

Conclusion: The outcome for children with incidentally found brain lesions is excellent. Close monitoring of these patients with serial MRIs may be a safe alternative to immediate biopsy and/or resection.

(POSTER 309)
PLEUROPULMONARY BLASTOMA: THE HOSPITAL FOR SICK CHILDREN EXPERIENCE
Mira Liebman, Ronald Grant, Glenn Taylor, Peter Kim, Paul Nathan.
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Background: Pleuropulmonary Blastoma (PPB) is a rare mesenchymal neoplasm. Published 5-year survival rates for types 1 (cystic), 2 (cystic-solid) and 3 (solid) are 85%, 60% and 45%, respectively. It has been suggested that type 1 is a precursor for types 2 and 3, and adjuvant chemotherapy +/- radiation has been recommended for all type 1’s regardless of resection status.

Objectives: To review the outcome of patients diagnosed with PPB at a large Canadian pediatric cancer center.

Design/Method: Retrospective chart review of PPB cases diagnosed at the Hospital for Sick Children between 1997-2009.

Results: Over this 13 year period, eight PPBs were diagnosed. Of these, four were type I, two were type II, and two were type III. Median age at diagnosis was 2 years 5 months (range 11 months to 14 years 11 months). The type I PPBs were treated with surgical resection alone, whereas types II and III received adjuvant therapy. Median follow up was 2 years 10 months (range 2 months to 7 years). All patients remained recurrence-free, with the exception of one patient with Type III PPB, who died of liver failure (Table 1).

Conclusion: This review of a single-institution experience suggests that type 1 PPB may be curable with surgical excision alone. We observed favorable outcomes after surgery and adjuvant chemotherapy/radiation in 7/8 patients with type 2/3 PPB. The role of chemotherapy and radiation for type 1 PPB requires further investigation.

(POSTER 310)
RHABDOMYOSARCOMA IN ADOLESCENTS AND YOUNG ADULTS AT NATIONWIDE CHILDREN’S HOSPITAL: A 25 YEAR REVIEW
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Background: Adolescents and Young Adults (AYA) with cancer are a group that has shown relatively weak improvements in survival compared to other age groups due to a relative lack of clinical trial participation, lack of adherence to prescribed treatments, and receiving medical care at inappropriate institutions. Indeed, studies have shown that patients in pediatric institutions have better overall survival and treatment-free survival rates when compared to adult institutions. AYA patients have also been noted to be less tolerant of pediatric treatment regimens. Prior studies have shown inferior survival for adults with rhabdomyosarcoma (RMS), but little study has been done specifically evaluating RMS in the AYA population.

Objectives: To review a single institution’s experience with RMS in the AYA age group as compared to younger patients with RMS with regard to patient characteristics at diagnosis, treatment outcome and treatment toxicities.
Conclusion: High level of MKP-1 expression may be associated with cisplatin resistance in neuroblastoma. Furthermore, a MKP-1 inhibitor increased cisplatin induced cell death, suggesting that high level of MKP-1 may contribute to cisplatin resistance in osteosarcoma cell lines. Since platinum has been widely used in the treatment of soft tissue sarcoma such as neuroblastoma, MKP-1 could potentially be a predictive biomarker in a broader base.

Objectives: This study was undertaken to examine the relationship of MKP-1 level and cisplatin resistance in neuroblastoma.

Design/Method: To determine the MKP-1 expression in neuroblastoma, we first examined the relationship of MKP-1 level and cisplatin resistance in neuroblastoma. MKP-1 expression was detected in all 3 neuroblastoma cell lines examined in the order of U2OS, SK-N-BE and SH-SY5Y from the highest to the lowest level. We then examined the cisplatin induced cell death by MTT assay. SH-SY5Y was not examined because the difficulty in evenly distributing the cells due to cell clumping. SK-N-BE is more resistant to cisplatin than SH-SY5Y, although less resistant than U2OS. Again, there is an inverse correlation between MKP-1 level and cisplatin sensitivity. In addition, pretreatment the cells with Triptolide, a known MKP-1 inhibitor, increased cisplatin induced cell death, suggesting that high level of MKP-1 may be associated with cisplatin resistance in neuroblastoma. Furthermore, MKP-1 expression in primary neuroblastoma tumor tissues from 9 patients was examined by real time RT-PCR and MKP-1 expressions in various level were observed.

Conclusion: High level of MKP-1 expression may be associated with cisplatin resistance in neuroblastoma cell lines, and MKP-1 expression in various level was observed in the neuroblastoma tumor tissues.

Background: Colorectal cancer (CRC) is the fourth-most common cancer with a high mortality rate. Familial adenomatous polyposis (FAP) is a hereditary form of CRC; FAP patients carry germline mutations of the tumor suppressor gene adenomatous polyposis coli (APC). However, the majority of sporadic adenomas and CRC have somatic APC mutations. The function of DLX genes in intestinal development has not been previously explored. The transcriptional regulation of Apo gene expression during development is unknown.

Objectives: We will assess DLX2 expression throughout intestinal development, identify the specific intestinal cell types in which DLX genes are expressed, and determine the intestinal phenotype of the Dlxs1/Dlxs2 double knockout mouse. We will determine DLX2 expression in rat ACF, intestinal polyps and tumors of Apomin mice, CRC cell lines and in CRC tumour specimens.

Design/Method: Using a chromatin immunoprecipitation (ChIP) assay of mouse intestinal mucosa, we will isolate regions of the Apo gene promoter bound to DLX2. We will characterize this DLX2 protein-Apo gene interaction using gel-shift and reporter gene assays, and assess APc expression in Dlx loss- and gain-of-function models using qRT-PCR and Western blotting.

Results: We have found that DLX2 is highly expressed in mouse embryonic and adult intestinal epithelia. Moreover, DLX2 is expressed in the carcinogen-induced rat ACF model as well as in some human colorectal cancer cell lines and tumors. Of particular interest, we have discovered that DLX2 is bound to the Apo gene promoter in vivo, co-expressed with Apo, and that there is decreased Apo expression in the Dlxs1/Dlxs2 mutant mouse with concomitant increased β-catenin and C-MYC expression. These results are consistent with activation of the Wnt-signaling pathway in the absence of Dlx gene function.

Conclusion: Dlx gene function may block the Wnt signaling pathway through up-regulation of Apo expression. Ultimately, restoring Apo expression may be a novel strategy towards preventing progression of intestinal polyps to adenocarcinoma. This research will contribute to our knowledge of the genetic and epigenetic regulatory pathways that control intestinal development, mucosal self-renewal and CRC.

Background: Many pediatric brain tumors, especially those of primitive neuroectodermal origin, demonstrate dose-dependent chemotherapy responses, making them appropriate targets for HDCT with AHSCR.

Objectives: We aimed to determine clinical outcome, potential prognostic variables, and toxicities for pediatric patients with various brain tumors treated with HDCT/ AHSCR.

Design/Method: Retrospective clinical review and outcome analysis were performed for 18 consecutive pediatric brain tumor patients treated with HDCT/AHSCR at UCLA from 1999-2009.

Results: Patients were 0.4-19 years at diagnosis (mean 5.6, median 2.3 y). There were 12 males and 6 females with 14 primary and 4 recurrent tumors: medulloblastomas (n = 7), supratentorial PNETs (n = 4), pineoblastoma (n = 1), ependymoma (n = 1), oligodendroglioma (n = 1), neurocytoma (n = 1) and non-germinomatous germ cell tumors (n = 3). Four patients had M2 disease, four had M3, and the remaining had M0. Extensive resection included 8 gross total resections (GTR) on first attempt, 1-GTR after recurrence, 6 subtotal resections, 2 of which were followed by GTR, and 3 without resection. Initial chemotherapy utilized various regimens. Consolidation regimens consisted of carboplatin (CP)/thiotepa (TT) (n = 8), CP/TP (cisplatin/VP) (n = 6), TT/VP (n = 2), cisplatin/cyclophosphamide (CPM)/ vincristine (n = 1), and CPM/melphalan (n = 1). Median time from diagnosis to transplant was 6 months (mean 1 yr). Nine patients received a single AHSCR, 8 underwent three and 1-four tandem AHSCR. A mean of 20 × 10^6 CD34+ cells/kg was infused per AHSCR (range 2 to 100 × 10^6). Neutrophil engraftment (ANC>500 × 2 days) was estimated by an average of 17 ± 8 (SD±) days post-transplant. Eight of the 18 patients(44%) received craniospinal radiation. However, of the 13 survivors, 9 avoided radiation therapy. All 10 patients with complete remission (CR) prior to AHSCR are alive and disease free, whereas 5 of 8 patients with partial remission (PR) or stable disease (SD) at the time of AHSCR have deceased, with the majority due to disease progression (one from therapy related toxicity). The probability for 3-year event-free survival (from first AHSCR) is 60.5% ± 16 (Mean ± Std. error) and for overall survival is 69.3% ± 11.5 (all patients). Evaluation of toxicity data is ongoing.

Conclusion: HDCT with AHSCR has a role in the treatment of pediatric brain tumors limited to selected patients with CR or PR prior to transplant. Currently, this modality is unlikely to salvage patients with SD.
Background: Pegfilgrastim comprises a 20 kDa polyethylene glycol molecule bound to the N-terminus of filgrastim. The elimination of pegfilgrastim is dependent on neutrophil-receptor binding, prolonging its systemic half-life and reducing the administration once per cycle. There are limited pediatric studies reporting the use of pegfilgrastim particularly on IC chemotherapy (Fox E. Clin Cancer Res 2009).

Design/Method: We retrospectively reviewed the medical records of patients with a diagnosis of sarcoma from October 2007 to October 2009 who received pegfilgrastim (~100 mcg/kg or 6 mg in patients > 50kg) administered subcutaneously in the outpatient setting 1 day after chemotherapy. We evaluated the data from cycle 1 to the beginning of cycle 6 chemotherapy (prior to local control therapy). We report demographic data, tumor characteristics, and episodes of ANC < 500 mm³, fever/ neutropenia admissions, number of PLT transfusions and cycles lengths and delays, and reported side effects of pegfilgrastim.

Results: 7 patients received alternating VAdC with IE every 2 weeks if minimum count recovery criteria were met (ANC > 750 and PLT > 75K). Median age was 14.8 years (6–19). 4 patients had localized Ewing/PNET and 3 patients had metastatic sarcomas (RMS, DSRCT and round cell sarcoma). 42 cycles of chemotherapy supported by pegfilgrastim were given prior to local control therapy. The average cycle length was 14.9 (14-16.2). Only 1 patient was delayed for neutropenia on 2 occasions (4.7%) and there were no delays for thrombocytopenia. Episodes of neutropenia (ANC < 500) between cycles were frequent (38%) but fever-neutropenia admissions were uncommon (3). Only 1 PLT transfusion was required. Leukocytosis (WBC > 15,000) was documented in 9.5% of cycles but no significant adverse events were reported. Current failure free survival is 100% at a median of 16.5 months (5-28 months).

Conclusion: Our preliminary experience supports the safety and efficacy of single dose pegfilgrastim in C – AYA sarcoma patients receiving interval compression chemotherapy.

(POSTER 315)

18FDG-PET FOR RESPONSE ASSESSMENT IN DESMOPLASTIC SMALL ROUND CELL TUMOR


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Background: Desmoplastic small round cell tumors typically have a large stromal component and often are extensively disseminated in the peritoneal cavity at diagnosis. These factors contribute to difficulty in quantifying response to chemotherapy using RECIST or WHO criteria. 18FDG-PET imaging is currently used to follow response to treatment in some patients with DSRCT, but no data has been reported on the utility of 18FDG-PET in this disease.

Objectives: To compare the overall disease response to three cycles of chemotherapy by 18FDG-PET and CT.

Design/Method: We conducted a retrospective chart review of eight patients with DSRCT who were imaged by 18FDG-PET and CT at diagnosis and after 3 cycles of chemotherapy. Response to chemotherapy was graded according to EORTC metabolic response criteria and RECIST.

Results: All patients were imaged by 18FDG-PET and CT prior to treatment. All patients received 3 cycles of high-dose cyclophosphamide, doxorubicin and vincristine and were re-imaged by both modalities after cycle 3. All tumors demonstrated some decrease in SUV (53% +/-20%) and longest diameter (21% +/-9%) with chemotherapy. The best response achieved by 18FDG-PET was a PR in 7 patients and by CT was a PR in 1 patient. Measured response was concordant between the 2 modalities in 2 patients.

Conclusion: In this small series response measurement by 18FDG-PET did not always correlate with response measurement by CT. A greater decrease in metabolic activity as compared to size was seen in all patients. Further studies are needed to define the role of 18FDG-PET in assessing early response of DSRCT to chemotherapy.

(POSTER 316)

SUNITINIB TREATMENT OF MALIGNANT PHEOCHROMOCYTOMA IN A PEDIATRIC PATIENT

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Background: Pheochromocytomas and paragangliomas are rare neuroendocrine malignancies in pediatric patients. In children, 30–40% are familial and nearly half are malignant. Surgical resection is the mainstay of therapy for non-metastatic disease. Metastatic disease requires systemic therapy; however, prognosis is poor due to a paucity of effective agents. Pheochromocytomas demonstrate hypoxia-inducible factor (HIF-1) which mediates upregulation of pro-angiogenic factors. Sunitinib, a tyrosine kinase inhibitor, interacts with multiple pro-angiogenic pathways including VEGF, c-KIT, RET, FLT-3 and PDGFR and may have efficacy in neuroendocrine tumors.

Objectives: We describe a case of rapidly progressive malignant pheochromocytoma and response to systemic therapy with sunitinib.

Design/Method: We provide clinical, histological, biochemical and radiographic details of a case of malignant pheochromocytoma and compare our data to the clinical presentations, biology and outcomes of the five other reported cases.

Results: A 12 year old male presented with fever, abdominal pain and left adrenal mass. Computed tomography and 18-FDG PET revealed elevated uptake (SUV~12) in left adrenal gland. Laparoscopic resection revealed a pheochromocytoma. Immunostaining was positive for chromogranin, Ki-67, c-KIT (faint), and negative for S-100, pankeratin, EMA, GFAP, inhibit and VEGF. Cyto genetics revealed loss at 1p and gain of chromosome 9 with no N-MYC amplification. Metastatic loci involved the abdominal cavity, liver, mesentery, humerus and thorax. Testing for RET, VHL, SDHB and SDHD gene mutations was negative. Systemic chemotherapy with cyclophosphamide, vincristine, dacarbazine, doxorubicin and thalidomide resulted in transient radiographic, biochemical and symptomatic response. Seven months later, he developed progressive disease. Sunitinib was initiated at 50 mg orally daily for 28 days every 6 weeks. He stabilized for four months based on biochemical, radiological and clinical symptoms. Hepatic metastatic lesions decreased by 20-30%. Other mesenteric masses also showed improvement. Free plasma normetanephrine decreased from 8.96 to 4.42 nmol/L. Adverse effects included Grade 3 neutropenia and thrombocytopenia. Tumor burden progressed, eventually resulting in his death.

Conclusion: Sunitinib was well tolerated and demonstrated activity in a case of aggressive metastatic pheochromocytoma. To our knowledge, this is the youngest patient to have intermittent improvement of pheochromocytoma with sunitinib. Further clinical studies of sunitinib for treatment of pediatric pheochromocytoma are ongoing.

(POSTER 317)

CENTRAL NERVOUS SYSTEM RHABDOID TUMORS: 10 YEARS EXPERIENCE

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Background: Rhabdoid phenotype is the diagnostic feature of CNS Atypical Teratoid Rhabdoid Tumors (ATRT), reported rarely in other CNS malignant tumors. CNS rhabdoid tumors (CNSRT) are highly malignant embryonal tumors of childhood associated with poor outcomes. Reported median overall survival (OS) after diagnosis in CNS ATRT patients is 11–17 months.

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PR, partial response; SD, stable disease.
Objective: To study presenting features, diagnostic work up, course of disease & treatment outcomes in children diagnosed with CNSRT at a regional pediatric cancer center.

Design/Method: Retrospective analysis of medical & tumor registry records from 1999 to 2009. Histopathology, immunohistochemistry & cytogenetic reports were reviewed to confirm the diagnosis. Survival functions were estimated using the Kaplan-Meier method. Survival by age at diagnosis was compared using the logrank test.

Results: Nine children were diagnosed with CNSRT. 5.7% of CNS tumors (n = 158). Seven were ATRT & 2 composite RT’s. All were female; median age at diagnosis was 40 months. Four were <3yrs & 5 were >3yrs of age. The most common presenting symptoms were vomiting, clumsiness followed by weakness, abnormal movements & eye deviation. Six patients had focal neurological signs & two had papilledema. Radiologically all tumors were large, heterogeneous, contrast enhancing with a variable presence of cystic components, necrosis, hemorrhage & calcification. The most common location was posterior fossa (79%). All had characteristic histopathology findings and immunohistochemistry. Loss of INI1 expression was shown in 7 patients & chromosome 22q abnormalities in 2 patients. Eight patients were treated with multimodality approach with surgery, chemotherapy and radiation. Gross total resection achieved in 7/8 patients. Initial remission achieved in 5/8 (62%), 2/8 (25%) were non responders & one patient had stable disease. Relapse or progression seen in 5/8 (62%). At 2 years from diagnosis overall mortality was 55%. Median event free survival was 11 months in all patients, in children aged < 3yrs it was 7 & 15 months respectively (p = 0.052). Median OS was 9 months in < 3yrs (cannot be estimated in >3yrs & all patients).

Conclusion: In spite of multimodality therapy CNSRT’s have poor outcomes with current therapeutic approach. Children younger than 3 years have worse prognosis. More research for targeted molecular therapy is needed for novel therapeutic strategies.

(Poster 318) NOVEL DRUG COMBINATION USING GEMCITABINE, DOCETAXEL AND BEVACIZUMAB IN RELAPSED AND REFRACTORY PEDIATRIC SARCOMAS

Pooja Hingorani, Frank Eshun, Rajive Shah, Thomas Walkiewicz, Andrea White-Collins, Masayo Watanabe

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Background: The outcome for patients with relapsed and refractory pediatric sarcomas remains dismal. Novel agents and drug combinations are needed to improve overall survival in these patients. The combination of gemcitabine and docetaxel has shown antitumor activity and improved progression free survival in patients with adult sarcomas and pediatric sarcomas such as Ewing sarcoma and osteosarcoma. This regimen is currently being evaluated in larger multi-center trials. Bevacizumab is a potent VEGF inhibitor that has antitumor activity as a single agent in sarcomas. The combination of paclitaxel with gemcitabine and bevacizumab was well tolerated in patients with metastatic breast cancer.

Objectives: To determine the safety and activity of gemcitabine, docetaxel and bevacizumab in patients with relapsed and refractory pediatric sarcomas previously treated intensely with other chemotherapeutic agents.

Design/Method: Three patients, two with multiply relapsed Ewing sarcoma and one with refractory undifferentiated sarcoma, were treated with the three drug combination. Patients received gemcitabine 900mg/m² on day 1 and day 8, docetaxel 100mg/m² on day 1 and day 8, and bevacizumab 5mg/kg on day 1 and day 15. The disease status was reevaluated at the end of every 2 cycles by imaging of the primary and metastatic sites. Therapy was stopped or changed at evidence of progression.

Results: Median age of the treated patients was 15 years. Median number of prior therapies was four. Median number of cycles administered was eight. One patient had stable disease and two patients had a partial response. The combination was well tolerated with the main toxicity being myelosuppression leading to grade 2–4 anemia, thrombocytopenia and neutropenia requiring transfusion and growth factor support.

Conclusion: These results suggest that gemcitabine, docetaxel and bevacizumab is feasible and might have activity in multiply relapsed and heavily pretreated pediatric sarcoma patients. The small number of patients limits our results and this combination should be evaluated in larger trials.

(Poster 319) CASE OF RHABDOMYOSARCOMA ARISING WITHIN A SACROCOCCYGEAL TERATOMA

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Background: Teratoma with malignant transformation (TMT) is defined as a somatic tumor arising within a teratoma. Somatic malignancies seen in TMT include adenocarcinoma, rhabdomyosarcoma, primitive neuroectodermal tumor, and leukemia. The vast majority of cases occur in adults and typically involve the gonads, although TMT of extragonadal sites have been reported. There are very few case reports of TMT occurring in children.

Objectives: To discuss a unique presentation and management options of TMT with rhabdomyosarcoma arising from a sacrococcygeal tumor in an infant.

Design/Method: We present a female infant delivered at 34 weeks gestation via Caesarean section with a large sacral mass diagnosed by prenatal ultrasound. On examination, the mass measured 50 cm in diameter and appeared to contain both solid and cystic components; the anus was displaced anteriorly.

Results: Imaging of the mass showed a type II sacrococcygeal tumor with both solid and cystic areas. AFP and HCG levels were markedly elevated consistent with germ cell tumor. Following near-total resection of the mass, pathologic review identified teratoma (both mature and immature components) with secondary rhabdomyosarcoma. Immunohistochemistry stains were strongly positive for desmin and smooth muscle actin. Ki-67 staining showed 70% positivity consistent with embryonal rhabdomyosarcoma. The rhabdomyosarcoma component was noted to be fully contained within the sacrococcygeal tumor demonstrating clear margins. Given the low risk classification of Stage II or III, Group 1 of the rhabdomyosarcoma fully encapsulated by the sacrococcygeal tumor, we decided to follow with close observation as the risks of giving chemotherapy +/- radiation outweighed potential benefits in this neonate. The patient has had no recurrence of disease 15 months after resection alone.

Conclusion: To our knowledge, this is the first case of rhabdomyosarcoma arising within a sacrococcygeal tumor. This case highlights management options of TMT with respect to this unique age group. Whereas studies in adults and older children show better prognoses treating TMT according to the transformed histology, chemotherapy/radiation administration in neonates has significant risks and co-morbidities. Further study in this area may be warranted to define further treatment options.

(Poster 320) QUALITY, READABILITY, AND SOCIAL REACH OF WEBSITES ON OSTEOSARCOMA IN ADOLESCENTS

Catherine Lam, Debra Roter, Kenneth Cohen

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Background: There is little understanding of the internet’s potential in promoting health communication and psychosocial support for adolescents with cancer. Scant information is known about the quality and readability of patient-oriented websites on many adolescent malignancies, including osteosarcoma.

Objectives: To determine the quality, readability, and potential social reach of a sample of English language websites on pediatric osteosarcoma.

Design/Method: Four osteosarcoma search terms were used in two leading search engines. For each search, the top 50 results were screened. Sites that were exclusively links, academic, duplicates, mainly non-informational or restricted-access were excluded. Quality ratings of the online health information were given according to the standard DISCERN instrument. Flesch Reading Ease and Flesch-Kincaid Grade Level were used to determine readability. Potential social reach was gauged by counts of social bookmarking/networking links; global estimated website traffic over 30 days and over 3 months; and availability of data specifically addressing adolescents.

Results: A total of 400 websites were assessed; 56 (14%) met inclusion criteria. Mean DISCERN quality rating of included websites was fair (49.8 on a 75-point scale); ratings ranged from 31.0 to 66.0 (poor to excellent, respectively). 45% classified as good/excellent. 86% of the websites were more difficult to read than recommended standards (mean Flesch Reading Ease 44.5, range 16.8–66.0; mean Flesch-Kincaid Grade Level 10.4, range 6.2–12.0). Social media links per website ranged from 0 to 226, including up to 5 site-specific unique social media. Rated websites received numerous visits in the past 30 days (50% received over 50,000 visits, with a combined estimate of 200,789,000 visits for the 56 sites). Only 12.5% of the websites specifically addressed adolescents.

Conclusion: Some patient-oriented websites on pediatric osteosarcoma are of acceptable quality; readability remains mostly at a level more challenging than recommended. Better awareness of the quality and types of internet resources for adolescent oncology patients is needed to promote health literacy and patient-provider communication. Studies are needed to understand the potential social reach of internet health information, along with patients’ use of these resources and their impact on adolescents’ informational and psychosocial needs.
Fifty-two patients were eligible. Comparing patients in which DNR was each eligible patient. Continuous variables were compared using Wilcoxon rank-sum groups on the basis of DNR status. The number of inpatient days, ICU days, and died between 2004 and 2008. Patients between 1 and 21 years with a histologically patients treated at Rainbow Babies and Children’s Hospital in Cleveland, Ohio, who We conducted a retrospective chart review of pediatric oncology performed during the last six months of life among pediatric oncology patients. Care in pediatric populations. Radiology costs and have fewer ICU stays. Extensive research into the benefits of Rainbow Babies and Children’s Hospital, Cleveland, Ohio, United States PEDIATRIC ONCOLOGY PATIENTS DNR STATUS AND HOSPITAL COST SAVINGS IN Pediatric oncology patients treated at Rainbow Babies and Children’s Hospital in Cleveland, Ohio, who died between 2004 and 2008. Patients between 1 and 21 years with a histologically confirmed cancer diagnosis at least six months prior to death were separated into two groups on the basis of DNR status. The number of inpatient days, ICU days, and invasive procedures performed during the last six months of life was determined for each eligible patient. Continuous variables were compared using Wilcoxon rank-sum tests. Results: Fifty-two patients were eligible. Comparing patients in which DNR was instituted (31, 60%) to patients in which DNR was not instituted (21, 40%), there was not a statistically significant difference in the median number of inpatient days (21 vs 22; p = 0.79), ICU days (1 vs 2; p = 0.73), or invasive procedures performed (3 vs 3 p = 0.87). However, comparing patients in which DNR was instituted >30 days prior to death (13 patients) to those in which DNR was not instituted revealed a nominal difference that trended toward statistical significance in median number of inpatient days (8 vs 22; p = 0.13) and ICU days (0 vs 2; p = 0.17). Conclusion: This pilot study demonstrated a trend toward a statistically significant association between the institution of DNR status in pediatric oncology patients and a reduction in hospital resources used in the last six months of life when DNR is instituted >30 days prior to death. Using DNR status as a surrogate marker for palliative care, these data support the design of a prospective analysis of the potential savings from pediatric palliative care programs. (POSTER 322) THE ROLE OF INTEGRINS AND CASPASE 8 IN THE PROGRESSION OF NEUROBLASTOMA Jennifer Willert, Natanya Maya, Dwayne Stupack, Robert Newbury UCSD/Rady Childrens, San Diego, California, United States Background: Despite significant advances in the treatment of pediatric malignancy, aggressive advanced stage metastatic neuroblastoma, particularly when associated with unfavorable biologic features such as amplified N-MYC oncogene, unfavorable histopathology, or normal ploidy, is generally associated with a poor prognosis. Favorable biology, age less than 18 months and lower stages of disease have an excellent chance for long term cure. Objectives: To analyze whether the expression of caspase 8 and integrins in neuroblastoma correlates with clinical behavior and patient outcome. Design/Method: We have analyzed 80 neuroblastoma patients in our extensive clinical data base treated at RCHSD/UCSD from 1996 to present. This database tracks prognostic features, relapse status and survival. Retrospective analysis was done for 1996-2006. We have prospectively enrolled patients from 2007 and forward. We anticipated continued enrollment through 2016 (100 patients expected). Fixed/frozen tissue and/or slides, and in some cases fresh tissue, have been sent to the Stupack lab on all but one of a total of 80 patients. Fifty-three of these tumors have been analyzed to date. Results: While our results are preliminary, we have seen a general trend as expected with lower stage, favorable biology neuroblastoma patients having tumors that express normal to high levels of caspase 8 and integrins. Likewise, infants less than 18 months of age, with high stage but favorable biology, who generally have excellent prognoses, have had normal to higher levels of caspase 8 and integrins. For aggressive higher stage patients, those patients who have maintained normal caspase 8 and integrin expression have tended to have more favorable biologic features and improved outcomes including long term survival and cure. Those patients with aggressive tumors and/or unfavorable biologic tumors have tended to have lower expression of caspase 8, but there was a full range of expression of caspase 8 in this subgroup of patients while integrins have mostly shown decreased expression. The implications of the findings are discussed. Conclusion: The expression of caspase 8 and integrins may correlate with clinical behavior of neuroblastoma. Agents that target these pathways and other interacting pathways may be considered for further testing and may be appropriate to consider as therapeutic agents in the future.
significant adverse events developed most-notably nausea and vomiting. Data in adults with disease response (SD, PR, CR) to front-line therapy suggested maintenance regimens were tolerable and increased progression free survival (PFS). Considering prolonged stable disease with developing toxicity, we began capecitabine-bevacizumab maintenance regimen. CT scans after 4 courses showed progression with increased omental wall thickening and peritoneal fluid. FOLFIRI-bevacizumab regimen was restarted. To date three additional courses have been completed with disease stabilization.

Conclusion: Our patient, 17 months after diagnosis, has maintained stable disease with excellent quality of life. Optimal treatment and duration of therapy for children with CRC is not well defined, but based on adult data. Continuing front-line regimen if tolerated and not initiating maintenance therapy may improve PFS and OS for patients with this typically highly aggressive disease. Toxicities, acute and cumulative, associated with prolonged treatment are not well known. Childhood CRC may be biologically different from adult-onset disease and may warrant pediatric focused clinic trials.

(PAPER 325)
SYNCHRONOUS PRIMARY NEUROBLASTOMA IN MONOZYGOTIC TWINS WITH DISTINCT PRESENTATION, PATHOLOGY, AND OUTCOME

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Background: Synchronous neuroblastoma can occur in monozygotic twins without twin-twin transmission. To date, eight sets of monozygotic twins with neuroblastoma have been reported and in most, twin- to- twin in utero metastasis is proposed as the mechanism of tumor development in the second twin based on metastatic disease in the absence of a primary tumor and/or later presentation. Synchronous neuroblastoma has not been described in this context.

Objectives: To present the clinical and pathological findings of a synchronous neuroblastoma in a pair of monozygotic twins without evidence of twin-twin transmission.

Method/Design: Case Presentation: Monozygotic twins conceived by in vitro fertilization and delivered at 33 weeks gestation developed neuroblastoma tumors that were morphologically, clinically and molecularly different, with a delay of 14 months between times of presentation. Twin 1 presented at 5 months of age with bilateral adrenal, thoracic paraspinal masses and liver metastasis without bone or bone marrow involvement. Pathology showed favorable histology, ploidy of 1, and MYCN amplification and low MKI. Twin 2 presented at 19 months of age, with right occipital mass, and multiple para-aortic retroperitoneal masses without adrenal involvement. Pathology showed poorly differentiated neuroblastoma, unfavorable histology with MYCN amplification, ploidy of 1.93 and high MKI. Both twins were treated according to protocol COG-A3973 for 6 cycles followed by surgery and high dose chemotherapy (carboplatin, melphalan, and etoposide) followed by autologous peripheral blood stem cell transplant. Twin A is alive and well with no evidence of disease while Twin B died of veno-occlusive disease complications during the post transplant period.

Conclusion: We propose synchronous primary tumors rather than twin-twin transmission based on the different ages of presentation, histology, ploidy, and tumor behavior.

(PAPER 326)
MULTIVISCERAL TRANSPLANTATION IN A PATIENT WITH UNRESECTABLE, RECURRENT HEPATOBLASTOMA

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Background: Primary malignant neoplasms of the liver account to 1% of pediatric tumors and hepatoblastoma (HB) represents 67% of hepatic malignancies. Complete resection either as initial approach or after chemotherapy, is the mainstay of treatment and is required for cure. Cisplatin-based chemotherapy has significantly rendered tumors resectable and dramatically improved the cure rate (80%). Liver transplantation is also a curative procedure for non-metastatic disease that remains unresectable after chemotherapy. “Primary transplantation” has shown similar success as conventional surgery. The only absolute transplant contraindication is the persistence of unresectable, extrahepatic metastases. Macroscopic venous invasion is not always considered a contraindication. Chemotherapy must be associated to liver transplant for a positive outcome.

Recurrent hepatoblastoma also requires complete resection for cure. In children with unresectable local recurrence, transplant is still considered an option. However, results are disappointing (30% cure).

Multivisceral transplant (MVT) can be considered as an option in selected cases when simple liver transplant is considered contraindicated.

Objectives: Describe a case of intrahepatic recurrent HB with unresectable disease, successfully treated with MVT and chemotherapy. Review of the literature.

Method/Design: Case report and systematic review of the literature.

Results: A 15-month ex-preemie was diagnosed with COG-stage III hepatoblastoma and treated with neoadjuvant VCR-3FU-CDDP followed by a partial hepatectomy and one cycle of adjuvant chemotherapy. Further treatment was held due to the development of volvulus requiring a large intestinal resection.

At 28-months of age the patient developed recurrence with unresectable masses throughout the liver. The portal vein could not be visualized and tumoral-thrombus was suspected. AFP was > 260,000. The metastatic work-up was negative. Trimetrexate then two cycles of CDDP/Doxorubicin were administered with drop of the AFP(232). A MVT of liver, pancreas, intestine and stomach was then performed. The postoperative course was uneventful, the AFP level normalized and the patient completed four cycles of adjuvant CDDP/Doxorubicin. Post-treatment evaluation has not revealed recurrence 3.5 years post transplant.

Conclusion: MVT can be considered as a therapeutic option in cases of intrahepatic recurrent HB with unresectable disease after chemotherapy. The addition of post-transplant chemotherapy is feasible and possibly influences outcome.

(PAPER 327)
WERNICKE’S-LIKE ENCEPHALOPATHY AND GENERALIZED ENCEPHALOPATHY FOLLOWING TREATMENT IN MEDULLOBLASTOMA

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Background: Encephalopathy has been described in patients with brain tumors following intrathecal chemotherapy and high dose systemic chemotherapy with autologous stem cell support. To the best of our knowledge generalized encephalopathy and especially one mimicking Wernicke’s encephalopathy has not been described.

Objectives: To report a case series involving children who developed clinical encephalopathy following completion of treatment for Medulloblastoma.

Method/Design: Retrospective review of children treated for Medulloblastoma using standard protocol and subsequently developed clinical encephalopathy at a single centre.

Results: Case One: An African American (AA) child was diagnosed at 5yrs of age. He had a gross total resection and was started on chemoradiation with 5cycles of Vincristine, radiation doses of 55.80Gy to posterior fossa and 23Gy to spinal cord. This was followed by maintenance chemotherapy. One year after this, he presented with Encephalitis and MRI-scans showed white matter changes. A year later, he presented with altered mental state that rapidly progressed to extensive cerebral and cerebellar injury with global atrophy on MRI-scans. He is now 17yrs and remains non-verbal, blind and unable to walk.

Case Two: 8 yr old AA was diagnosed at 5yrs of age. She had subtotal resection followed by chemoradiation consisting 5cycles of induction with Vincristine, standard dose of 23Gy cranio-spinal and 54Gy to posterior fossa. She then received maintenance chemotherapy with Vincristine, Cisplatin, Lomustine and Cyclophosphamide. 6 months from completing her radiotherapy, she had rapid neurological decline with MRI showing patchy enhancement within the Pons. She received hyperbaric oxygen therapy with some improvement.

Case Three: 20yr old Caucasian female diagnosed at 19yrs of age. After subtotal resection, she was enrolled into the current high risk protocol and received chemoradiation with Vincristine, 36Gy of cranio-spinal and total of 55.80Gy. She was then randomized to 6 cycles of Isotretinoin, Cisplatin, Cyclophosphamide and Vincristine. 3weeks into completing maintenance, she had a rapid neurological decline. MRI showed Wernicke’s-like encephalopathic changes.

Conclusion: The exact mechanism involved in producing the clinical encephalopathy and MRI changes described is still unclear. However, these cases highlight the importance of being intuitive in children who have received treatment for medulloblastoma presenting with neurological problems.

(PAPER 328)
GLIOMATOSIS CEREBRI IN CHILDREN—A MULTIFACETED DISEASE

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Background: Gliomatosis cerebri (GC) is a rare entity in the central nervous system, defined as a diffusely infiltrative glial neoplasm without a distinct mass...
lesion. Less than 50 cases are described in the pediatric literature with poor outcome.

**Objectives:** Challenges in the management, rationale for treatment and outcomes need to be discussed.

**Design/Method:** We report two cases of children with GC who were treated at our centre, with differing clinical presentations and outcomes.

**Results:** The first case is an eight year-old boy with sudden onset status epilepticus and speech difficulties. MRI revealed an infiltrative mass within the left frontal and temporal lobes, insular cortex and left hippocampus. Biopsy showed a diffuse infiltrative glioma, WHO III. The second case, a nine year-old boy, presented with a one month history of facial droop and a three month history of cognitive decline, deteriorating attention span, and learning disability. MRI revealed a infiltrative mass involving the left frontal lobe, corpus callosum, internal capsule, insular cortex and the contralateral cingulate gyrus. Biopsy showed infiltrative glioma with diffuse desmoplasia, the latter usually predicting a lower grade neoplasm.

Both patients were started on oral Temozolomide (TMZ) and tolerated it well with no toxicity. Patient 1 received twelve cycles as his MRI remained stable. Seizure control was only achieved on multiple anticonvulsive drugs. One year after diagnosis, he showed clinical and radiological deterioration, and palliation was initiated. He remains well eighteen months post-diagnosis with good quality of life. Patient 2 progressed rapidly neurologically and radiologically on treatment, and TMZ was stopped after 4 cycles. He underwent left frontal lobectomy for seizure control and debulking as he presented unresponsive and in status epilepticus. He never regained consciousness and died 6 months after diagnosis.

**Conclusion:** Pathology and location are not predictive of outcome in these cases, although cognitive decline as primary clinical presentation predicted a worse outcome. Treatment is difficult and should be guided by quality of life, as long term survival is not reported yet. Quality of life depends on the extent of neurological deficits secondary to CNS tumor invasion. Further international observation and treatment studies are required.

**(POSTER 329)**

**RISK FACTORS FOR WILMS TUMOR**

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**Background:** The established risk factors for Wilms Tumor (WT) include certain congenital anomalies and genetic conditions. However, the data is conflicting with regard to environmental risk factors for this highly aggressive tumor. Objectives: We investigated the relationship between potential risk factors and their association with WT.

**Design/Method:** A hospital-based, case control study was conducted with 65 patients (33 WT patients and 32 matched controls) ages 3 months to 19 years. Exposure information was collected from parents of case patients and their matched controls through interviews. Information pertaining to over 200 exposure variables was collected. Odds ratios (OR) were derived from unconditional logistic regression models.

**Results:** A family history of lung disease (OR = 3.57; 95% Confidence Interval [CI] = 1.15 to 11.1) was found to increase the risk of WT. Interestingly, several protective factors were detected. There was a decrease risk of WT in children who were exposed to cats and horses (OR = 0.01 to 0.80, respectively). Additionally, children who received the measles-mumps-subella (MMR) vaccine were less likely to develop WT (OR = 0.33; 95% CI = 0.12 to 0.92). Children who consumed cured meat in the first year of life also had a decreased risk of WT (OR = 0.34; 95% CI = 0.13 to 0.94). No maternal or paternal risk factors were detected. We were unable to confirm earlier reports of various factors identified as increasing the risk of this tumor (high birth weight, anesthesia during birth, neonatal jaundice, maternal consumption of coffee and tea, maternal smoking, maternal hypertension, radiation, household and garden pesticides).

**Conclusion:** We found that a family history of lung disease increases the risk of WT, but many risk factors previously suggested have no association. The hygiene hypothesis may explain why certain factors decreased the risk of WT (exposure of the child to horses and cats and the MMR vaccine) and warrants further investigation. Extension of this study to a larger sample size of patients will also provide clarification of these observations.

**(POSTER 330)**

**IFOSFAMIDE INDUCED NEUROPSYCHIATRIC TOXICITY IN PEDIATRICS**

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**Background:** Ifosfamide is a chemotherapeutic agent used in treatment of sarcomas and hemato logic malignancies. Ifosfamide produces unique neurotoxicity that may consist of confusion, seizures, hallucinations, incontinence, and cranial nerve abnormalities. Approximately 5-30% patients undergoing therapy experience encephalopathy. This phenomenon has been widely reported in adult literature, but less frequently in the pediatric population. The greatest known risk factor for neurotoxicity is hypobulinemia; others include renal dysfunction, low total bilirubin, low hemoglobin, and concurrent administration of other neurotoxic agents.

**Objectives:** We report two cases of ifosfamide-induced encephalopathy in adolescents undergoing treatment for osteosarcoma.

**Design/Method:** We present two adolescent patients who experienced encephalopathy following ifosfamide treatment for lower extremity osteosarcoma. In the first case, encephalopathy was manifested by rarely reported psychiatric disturbances. The second case consisted of the more commonly reported symptoms of ifosfamide induced neurotoxicity.

**Results:** Both adolescents experienced ifosfamide encephalopathy following first dosage of the drug. Both also had hypobulinemia on day of administration. The first patient, a 16 year old male, suffered from biparietal and oppositional defiant disorder which had been controlled with lithium. Within 48 hours post-ifosfamide administration, he experienced agitation, hallucinations, aphasia and incontinence. The second patient, a 15 year old female, experienced hand tremors, disorientation, auditory hallucinations and incontinence over the same time span. Both were treated with methylene blue, the standard treatment according to the literature. The first patient responded immediately; the second required increased frequency of dosage before response was seen. Both returned to baseline neurologic status within 96 hours of treatment with methylene blue. The first patient completed osteosarcoma therapy with ifosfamide and pre-medication with methylene blue without experiencing further episodes of encephalopathy. The second patient did not complete her initial course of ifosfamide, and continuation of this therapy is under current discussion.

**Conclusion:** As evidenced by our two patients, pediatric presentation of ifosfamide neurotoxicity is variable. It is necessary to be aware of multiple manifestations of adverse effects of this drug. Of equal importance is thorough assessment of the pediatric patient’s risk factors prior to ifosfamide administration. Further research is recommended to determine whether psychiatric history provides predisposition to ifosfamide encephalopathy.

**(POSTER 331)**

**SECRETORY CARCINOMA OF THE BREAST: A PEDIATRIC ONCOLOGY PERSPECTIVE**

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**Background:** Breast cancer is extremely rare in children, accounting for less than 1% of all childhood malignancies. Secretory breast carcinoma (SBC) is an indolent tumor with the capability of metastasizing. SBC has been reported in males and females in both the pediatric and adult population. There is no consensus on optimal treatment modalities, constituting a challenge for the pediatric oncologist.

**Objectives:** We present a new pediatric case, with special emphasis on the management.

**Design/Method:** Case report and review of the literature.

**Results:** A 12 y girl previously healthy presented with a two month history of a non-tender right breast lesion involving the nipple and areola complex. Review of systems was negative except for the breast lesion. Ultrasound demonstrated a well-circumscribed hypoechoic solid mass measuring 1.5 x 1.7 x 2.4 cm. An ultrasound core biopsy reported an invasive carcinoma of the breast, which prompted a right mastectomy plus sentinel lymph node biopsy. The pathology revealed a SCB with surgical margins and sentinel lymph node both negative for malignancy. Estrogen and progesterone receptors were positive. She received no further treatment and has remained well with no evidence of recurrence after seven months.

**Conclusion:** Although rare, SCB is the most common breast cancer in children with 40 cases reported since it was first described in 1996. The biology of the tumor has shown it to be indolent in nature with one reported pediatric fatality 12 years after diagnosis. Therefore, most reports suggest, initial treatment is surgical with wide excision or quadrantectomy. In premenarchal patients the surgeon should make an attempt to preserve the breast bud if possible without jeopardizing local control. Sentinel lymph node biopsy, offering a lower complication rate, should be performed for staging as several reports have shown lymph node involvement. The role of hormonal therapy and radiation therapy remain to be established but do not appear to offer advantage for localized disease. In metastatic disease SCB is relatively unresponsive to chemotherapy. The recent description of a balanced t(12;15)(p13;q25) translocation which creates a ETV6-NTRK fusion gene, encoding a chimeric tyrosine kinase, offers a new target for drug development for metastatic disease.
(POSTER 332)
NEPHROTOXICITY COMMON WITH OSTEOSARCOMA CHEMOTHERAPY

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Background: Standard therapy for osteosarcoma incorporates multiple nephrotoxic agents, including cisplatin, methotrexate and ifosfamide. The reported incidence of renal injury during therapy for osteosarcoma is highly variable. Nephrotoxicity is often defined as an increase in serum creatinine more than 1.5 times the upper limit of normal. However, it is well established in the literature that changes in serum creatinine do not occur until at least 50% of renal function is impaired. Therefore, less severe nephrotoxicity is likely underappreciated.

Objectives: To characterize the incidence and degree of nephrotoxicity during osteosarcoma therapy.

Design/Method: A 10 year retrospective analysis of 47 patients diagnosed with osteosarcoma was performed (1996–2006). Records were analyzed for patient variables including age, gender, race, body mass index, cumulative cisplatin dose, ifosfamide exposure, as well as baseline and intra-therapy serum creatinine. Groups were compared using the Wilcoxon signed rank test (p < 0.05 significant).

Results: During the course of therapy 79% of patients had a transient increase of at least 25% in serum creatinine. Patients with a transient increase had a significantly higher maximum creatinine during the first 10 weeks of therapy and at the conclusion of therapy compared with patients who did not develop a transient increase in creatinine (p < 0.05). No other variables were significantly different between these groups.

Conclusion: Chemotherapy for osteosarcoma may lead to nephrotoxicity. The majority of patients studied had at least a transient increase in serum creatinine during therapy. The long term implications of this degree of nephrotoxicity are unclear. Furthermore, it is not known whether these patients have an increased risk for future renal dysfunction. Long term follow up of renal function is necessary, and the development of more sensitive methods for detecting nephrotoxicity is mandated.
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