

**Society for Pediatric Pathology
Spring Meeting – San Antonio, Texas
February 26-27, 2011**

Abstracts are listed in presentation order, beginning with Platform Presentations.

Platform Presentations

1 Immunohistochemical Expression of Embryonic Stem Cell Markers in Malignant Rhabdoid Tumors of the Kidney

J Deisch, J Raisanen, D Rakheja, UT Southwestern Medical Center, Dallas, TX; Children's Medical Center, Dallas, TX.

Background: Malignant rhabdoid tumor (MRT), a highly aggressive pediatric neoplasm, is molecularly characterized by inactivating mutations of SMARCB1, a potent tumor suppressor and a member of the SWI/SNF chromatin remodeling complex. It has been suggested that oncogenesis in SMARCB1 deficient cancers is driven not by the loss of SWI/SNF function but by an aberrant functioning of the BRG1-containing SWI/SNF complex. Since Brg1 is required for self-renewal and pluripotency of mouse embryonic stem cells, we hypothesized that the human MRTs may express pluripotency genes such as SALL4, LIN28, and OCT4.

Design: Immunohistochemistry (IHC) for SALL4 (Abnova Corporation), LIN28 (Protein Tech Group), and OCT3/4 (Santa Cruz Biotechnology) was performed on representative sections of 5 MRTs of the kidney (MRTKs). IHC was performed on Ventana BenchMark XT autostainer using standard immunoperoxidase techniques. Appropriate positive and negative controls were used. Nuclear staining for SALL4 and OCT3/4 and cytoplasmic/nuclear staining for LIN28 were considered positive. The staining intensity was graded as absent, weak, moderate, or strong. The percentage of positively staining tumor cells was graded as 1+ (>0 and <30%), 2+ (>30 and <60%), 3+ (>60 and <90%), or 4+ (>90%).

Results: The patients, 3 males and 2 females, ranged in age from 5 to 17 months (mean 9 months; median 12.5 months). The tumors ranged in size from 1.2 cm to 9 cm (mean and median 5.1 cm). By pathologic exam, 2 (40%) tumors were stage I, 2 (40%) were stage II, and 1 (20%) was stage III. Four of 5 (80%) tumors showed robust expression of SALL4 and LIN28, but no tumor showed any expression of OCT3/4. SALL4, LIN28, and OCT3/4 were not expressed in the non-lesional kidney.

Conclusion: Our study is the first to demonstrate the immunohistochemical expression of embryonic stem cell markers, SALL4 and LIN28, in MRTKs. Our results suggest that MRTKs may arise from and share features with embryonic stem cells. This may explain the primitive appearance and polyphenotypic immunoprofile of the MRT cells. It has been suggested that the LIN28-let7 regulatory network may be a novel target for overcoming therapeutic resistance to ionizing radiation in cancers with activated Ras signaling. Since constitutive activation of Ras/PI3k/Akt pathway has been suggested to induce an anti-apoptotic state in MRTs, our demonstration of LIN28 immunoexpression is the first

step toward identification of LIN28 as a therapeutic target in MRTs.

2 Evidence of mTOR Signaling in Childhood Ependymoma - A Potential New Therapeutic Intervention

LR Margraf, DC Bowers, CJ Horton, J Brugarolas, D Rakheja, UT Southwestern Medical Center, Dallas, TX; Children's Medical Center, Dallas, TX.

Background: Mammalian target of rapamycin (mTOR) cell signal pathway plays an important role in many cancers and therapy with rapamycin and its analogs have shown activity against these malignancies. Little is known of mTOR pathway involvement in ependymomas. We recently cared for a 4 year old boy with a large, incompletely resected, progressive, fourth ventricular ependymoma (WHO grade 2), which was refractory to standard therapy. Subsequently, he received once daily therapy with rapamycin and showed a near complete response that was sustained for 18 months.

Design: We examined histologic tumor sections from this child and 14 other children with WHO Grade 2 ependymomas for immunohistochemical expression of phosphorylated (Ser235/236) S6 ribosomal protein (pS6) (rabbit monoclonal, Cell Signaling Technology, MA), a marker of mTOR complex 1 activation. Primary cell lines derived from five of these tumors were treated with a range of therapeutic doses of rapamycin and the effects on cell viability were examined by manual cell counts after staining with the vital dye trypan blue. Results were compared to the mTOR activated cell line HEK-293T/17 (ATCC, Manassas, VA), similarly treated with rapamycin, as a control.

Results: The tumor from the index patient and 11 of the other 14 ependymomas showed at least focal (>10%) cytoplasmic staining for pS6 in tumor cells by immunohistochemistry. Staining was accentuated in areas of neoplastic ependymal surface growth. Tumor cell line cell viability at 48 hours was reduced by 65-85% in the three pS6-positive tumors treated with 15-20 ng/ml rapamycin compared to 50% reduction in viable cells in the control HEK-293T/17 cell line and 40% reduction in the two pS6-negative cell lines treated with the same doses of rapamycin.

Conclusion: These results strongly suggest that mTOR pathway signaling plays a role in childhood ependymoma biology. Further investigation of potential biomarkers and targets of the mTOR pathway is warranted to help treat this challenging brain malignancy.

3 Wilms' Tumor with Favorable Histology: A Deletion of NTRK3 Gene on Chromosome 15q Identified Using SNP Copy Number Arrays

SC Shulman, HM Katzenstein, W Tang, F David, M Bouzyk, MR Rossi, CA Abramowsky, SM Langness, BM Shehata, Children's Healthcare of Atlanta, Atlanta GA; Emory University School of Medicine, Atlanta GA.

Background: Wilms' tumor is characterized by an abnormal proliferation of primitive embryologic cells of the kidney. Although many genetic studies of Wilms' tumors have been performed, to date, loss of heterozygosity (LOH) on chromosomes 1p and 16q are the only markers that significantly increase risk of relapse and death. Using a high resolution SNP copy number array, we chose to analyze Wilms' tumors for additional novel changes that may be predictive markers of the clinical course of these tumors.

Design: We reviewed over 300 cases of renal tumors from 1975-present from Children's Healthcare of Atlanta. We found over 70 cases of blastema predominant Wilms' tumor and reviewed all subsequent histologic, demographic and clinical data. Additionally, frozen tissue from 5 of the cases were analyzed using Illumina Human Omni1_Quad SNP Arrays to determine copy number and LOH events.

Results: Analysis of the five tumors displayed characteristic Wilms' tumor LOH of chromosomes 1p, 11p, 11q and 16q. In one of the 5 tumors analyzed, LOH of 15q25.3 (chr15:84,557,249-86,799,002, hg18), which includes the NTRK3 gene was detected and has remained in remission for 6 years since their initial treatment. This region would not have been identified by standard cytogenetic analysis.

Conclusion: Previous reports of ETV6-NTRK3 translocations have been well documented in the literature, although none have specifically mentioned deletion of the NTRK3 gene. The ETV6-NTRK3 translocation results in over expression of the NTRK3 receptor tyrosine kinase, and has been previously associated with unfavorable outcomes in Wilms' tumors. In patients with LOH of 15q, there will be down regulation of the NTRK3 gene and, as such decreased expression of the receptor tyrosine kinase. This suggests that LOH of 15q25.3 is associated with histologically favorable tumor and a better outcome as seen in our patient. This data suggests that further studies are warranted to determine if there is a positive correlation between loss of 15q25.3 and disease outcomes in Wilms' tumor.

4 Neuroblastoma, Undifferentiated Subtype: A Report from the Children's Oncology Group

Larry L. Wang¹, Rie Sukanumal, Jason P. Tovar¹, Arlene Naranjo², Wendy B. London², Michael D. Hogarty², Julie M. Gastier-Foster², A. Thomas Look², Julie R. Park², John M. Maris², Susan L. Cohn², and Hiroyuki Shimada^{1,2}, ¹Department of Pathology and Laboratory Medicine, Childrens Hospital Los Angeles and University of Southern California Keck School of Medicine, and ²Children's Oncology Group, Neuroblastoma Studies.

Background: After being defined in the International Neuroblastoma Pathology Classification in 1999, little was known and reported about histologic and clinicopathologic features of Neuroblastoma, Undifferentiated subtype (NB-UD).

Design: Review of histopathologic features of NB-UD, archived at the COG Neuroblastoma Pathology Reference Laboratory, Childrens Hospital Los Angeles, was conducted and the results were analyzed along with their clinical and biological characteristics.

Results: During the period 6/1/2001 to 9/30/2010, 5,156 peripheral neuroblastic tumors were reviewed and 146 cases (2.8%) were diagnosed as NB-UD by central review at the Pathology Reference Laboratory. Pathology slides from 132 of the 146 NB-UD tumors were banked and available for detailed review. NB-UD was characterized by densely packed tumor cells without clearly recognizable neuropil. Tiny foci of necrosis, fibrin and/or collagen deposition frequently imposed difficulty in distinguishing them from neuropil. Tumor cells in NB-UD typically had one or few prominent nucleoli (107/132, 81%), whose presence was significantly related to MYCN amplification in the tumors ($p < 0.013$). Immunohistochemically, NB-UD typically contained TH (tyrosine hydroxylase) positive cells sporadically distributed in the tumor tissue. Clinically more than 2/3 of patients were between 1.5 and 5 years of age, and the majority (120, 82%) had distant metastases at the time of diagnosis (INSS stage 4 disease). NB-UD histology was associated with the adverse prognostic factors, such as MYCN amplification (117/138, 85%) and diploid DNA content (82/131, 63%). Among these 146 cases, 141 patients were available for survival analyses. The overall 3-year EFS and OS were 48.7% \pm 5.7% and 53.4% \pm 5.7%, respectively. Age (<18 months vs. >18 months), INSS clinical stage (stage 4 vs. not stage 4), and ploidy (hyperdiploid vs. diploid) were not significantly associated with outcome. However, EFS and OS for patients with MYCN amplified tumors (53.3% \pm 6.2%, 56.6% \pm 6.3%) were significantly better than for patients with MYCN non-amplified tumors (32.9% \pm 15.6%, 28.8% \pm 14.0%) with p-values of 0.0138 and 0.0111, respectively.

Conclusion: Among the peripheral neuroblastic tumors, NB-UD makes a unique group of rare and highly aggressive tumors regardless of age, clinical stage, and ploidy pattern.

5 19q13.4 Loss of Heterozygosity and Occult Androgenetic/Biparental Mosaicism in Sporadic Hepatic Mesenchymal Hamartoma

J Lin, B Cole, X Qin, M Zhang, R Kapur, Seattle Children's Hospital and University of Washington, Seattle, WA; Jinling Hospital and School of Medicine, Nanjing University, Nanjing, Jiangsu, China.

Background: Hepatic mesenchymal hamartoma (HMH) is associated frequently with rearrangements of chromosome 19q13.4. A previous study demonstrated androgenetic/biparental mosaicism (ABM) in an infant with HMH and placental mesenchymal dysplasia (PMD). The androgenetic cells in ABM have pan-genomic

paternal uniparental disomy and loss of heterozygosity (LOH) at every chromosomal locus. We hypothesize that occult ABM or other causes of 19q13.4 LOH underlie cases of sporadic HMH with no recognized history of PMD or other ABM-related conditions.

Design: 11 patients with HMH were identified retrospectively from the surgical pathology records of a pediatric hospital between 1962 and 2009. DNA was extracted from archival formalin-fixed, paraffin-embedded HMH tissue. LOH at 19q13.4 was detected by sequencing 6 single nucleotide polymorphisms (SNPs) and methylation-specific PCR (MS-PCR) amplification of the PEG3 gene, which undergoes parental imprinting (maternal methylation). Quantitative analysis of 10 short tandem-repeat (STR) microsatellite polymorphisms (AmpliFistR Profiler Plus) was performed to identify ABM based on an imbalance of paternal and maternal alleles at multiple chromosomal loci.

Results: 19q13.4 LOH was demonstrated in 2 of the 11 hamartomas by SNP and/or MS-PCR. In the 2 affected tumors, analysis of informative SNPs showed a marked excess of one allele over the other and PEG3 MS-PCR demonstrated excess representation of the paternal unmethylated allele. STR analysis revealed a consistent imbalance of paternal and maternal alleles at 10 different chromosomal loci, indicative of ABM, in 1 of the 2 tumors with 19q13.4 LOH. These genetic changes were greatest in extracts from stroma-rich, as opposed to epithelium-rich, portions of the hamartomas. Retrospective review of histology and available clinical records from the 11 patients revealed no significant differences apart from the fact that the patient with occult 19q13.4 LOH and ABM had numerous cutaneous hemangiomas.

Conclusion: Sporadic HMH may be a consequence of diverse genetic pathogenetic mechanisms. Our molecular genetic results from 11 HMH cases indicated that occult ABM underlies at least one of the 11 cases and suggest that in such instances androgenetic cells localize primarily in the stroma of HMH tissue. In addition to other types of cytogenetic abnormalities of the 19q13.4 site, LOH due to ABM or other genetic alterations may play a pathogenic role of sporadic HMH, but additional research is needed to determine the frequency and importance of these changes. Awareness of the association of ABM and HMH is important because the presence of androgenetic cells in other tissues imposes a potential risk for other disorders associated with paternal uniparental disomy.

6 Morphoproteomics Provides Support for Dysmaturation and Immune Dysregulation in the Pathogenesis of Osteolytic Langerhans Cell Histiocytosis

S Alexandrescu, N Tatevian, BA Czerniak, M Covinsky, N Burns, RE Brown, Department of Pathology, University of Texas at Houston - Health Science Center; Department of Pathology, MD Anderson Cancer Center.

Background: Langerhans histiocytosis (LCH) has a challenging and still unclear pathogenesis. A body of literature points to impaired maturation of the lesional

dendritic cells, and to immune dysregulation in the form of increased FOXP3 cells. Various cytokine abnormalities (expression of transforming growth factor (TGF β)) have been reported, as well as abnormalities in lipid content in LCH cells. Morphoproteomic techniques were applied to identify the signal transduction pathways that could influence maturation and immune regulation in osteolytic LCH.

Design: Five pediatric cases of osteolytic LCH were examined, using antibodies against CD1a, S100, CD68, CD8, FOXP3, phosphorylated (p)-STAT3 (Tyr705), protein kinase C (PKC) (α), phospholipase (PL)D1, fatty acid synthase (FAS) and zinc finger protein, Gli2. Positive and negative controls were performed. A FOXP3(+)/CD8(+) cell ratio was calculated by counting the FOXP3 and CD8(+) cells in 10 high power fields for each case. The percentage of cells with nuclear Gli2 was determined using an automated scoring system.

Results: There is induction of sonic hedgehog (SHH) mediators consistent with TGF β signaling pathway through Smad3-dependent activation of Gli2, findings supported by the plasmalemmal and cytoplasmic expression of PKC α and PLD1, and nuclear expression of Gli2, in the majority of lesional cells. FOXP3(+)/CD8(+) cell ratio is increased, ranging from 13.1/1-1/1. There is moderate cytoplasmic expression of FAS in most of the Langerhans cells and in the osteoclastic component.

Conclusion: With our study, we strongly suggest that TGF β cell signaling pathway is a major player in the pathogenesis of LCH, leading to induction of the SHH pathway in the form of nuclear Gli2 expression and thereby, impaired maturation of LCH histiocytes. It could also cause a state of immune frustration in LCH, by inducing CD4(+)/CD25(-) cells to transform into CD4(+)/FOXP3(+) cells. This coincides with the clinical evidence of a response to thalidomide in most patients with osteolytic LCH, given its reported ability to reduce TGF β 1 and FOXP3 cells. Such signaling, and the presence of FAS in the majority of cells, has potential therapeutic implications. Metformin targets the TGF β pathway and inhibits FAS. It also down regulates the expression of Gli2. Metronomic doses of cyclophosphamide inhibit T regulatory cells preferentially.

7 The Tumor Suppressor Protein Tyrosine Phosphatase, Epsilon (PTPE) May Play a Role in Wilms Tumorigenesis

D Rakheja, N Fustino, S Khokhar, JF Amatrua, UT Southwestern Medical Center, Dallas, TX; Children's Medical Center, Dallas, TX.

Background: Wilms tumor, a primitive multilineage malignant neoplasm, is often associated with and presumed to arise from persistent foci of embryonic renal tissue called nephrogenic rests. However, the early molecular events that orchestrate the putative progression of nephrogenic rest to Wilms tumor are unknown. Protein tyrosine phosphatases, in concert with protein tyrosine kinases, regulate the structure and function of proteins involved in signaling pathways such as those involved in the control of cell proliferation, adhesion, and migration.

Protein tyrosine phosphatase, epsilon (PTPE) is a potential tumor suppressor that is normally expressed in the mature kidney. However, its role in renal development and Wilms tumorigenesis has not been studied.

Design: Immunohistochemistry (IHC) was performed on formalin-fixed/paraffin-embedded tissue microarray sections of 51 Wilms tumors, 6 nephrogenic rests, and 13 fetal renal cortices spanning 15 to 39 weeks' gestation. The tissue microarrays contain cores of non-neoplastic postnatal renal cortices as controls. IHC was performed on Ventana Discovery autostainer using anti-PTPE antibody (Sigma-Aldrich) and standard immunoperoxidase techniques. Appropriate controls were used. The staining intensity was graded as absent, weak, moderate, or strong. A note was made of the type of cells that stained positive. SYBR Green-based quantitative real-time PCR (qPCR) assay for PTPE mRNA expression in 12 frozen Wilms tumors and 2 non-neoplastic renal cortices was performed on ABI 7500 (Applied Biosystems) using primers and reagents purchased from SA Biosciences. β -actin and GAPDH were used to normalize expression, which was calculated by the delta-delta Ct method.

Results: In the developing fetal renal cortex, strong PTPE immunostaining was noted in blastema and primitive epithelial structures at 15 weeks' gestation, after which the staining intensity variably decreased to moderate intensity in tubular epithelial cells and glomerular mesangial cells, which was the pattern seen in the postnatal renal cortices. Strong staining for PTPE was seen in 6 of 6 (100%) nephrogenic rests. Of the 51 Wilms tumors, PTPE staining in tumor cells was absent in 2 (4%), weak in 26 (51%), moderate in 16 (31%), and strong in 7 (14%). Strong endothelial staining for PTPE was seen in all cases and served as internal positive control. 8 of 12 (67%) Wilms tumors showed more than/equal to 10-fold decreased expression of PTPE mRNA compared to the average expression in 2 control renal cortical tissues.

Conclusion: Our study suggests that decreased expression of the tumor suppressor gene PTPE may play a role in Wilms tumor biology, particularly in the progression of nephrogenic rests to Wilms tumors. Further exploration of the signaling pathways affected by PTPE may lead to the discovery of potential therapeutic targets.

8 Immunohistochemical Expression of NOTCH Pathway Proteins in Hepatoblastomas

D Rakheja, JB Litten, TT Chen, S Khokhar, R Schultz, GE Tomlinson, UT Southwestern Medical Center, Dallas, TX; Children's Medical Center, Dallas, TX; UT Health Science Center, San Antonio, TX.

Background: NOTCH signaling, implicated in regulation of cell fate, involves cell-cell interaction between NOTCH-family of transmembrane receptors (NOTCH1, NOTCH2, NOTCH3, NOTCH4) and their transmembrane ligands (JAGGED1, JAGGED2, DLL1, DLL3, DLL4). Ligand binding leads to 2 proteolytic

cleavages in NOTCH receptors: the first catalyzed by ADAM-family metalloproteases and the second by γ -secretase complex that contains Nicastrin, Presenilin, PEN2, and APh1. The latter cleavage produces Notch intracellular domain (NICD) that translocates to the nucleus to promote transcription of HES- and HEY-family genes. NOTCH2 is known to delay hepatoblast maturation in early hepatic organogenesis and the reduction of NOTCH2 expression correlates with differentiation of hepatoblasts into hepatocytes and biliary cells. The neoplastic cells that comprise hepatoblastoma resemble the developing liver. Therefore, we hypothesized that NOTCH2 is involved in hepatoblastoma tumorigenesis.

Design: Immunohistochemistry (IHC) for NOTCH2 was performed on 24 hepatoblastomas retrieved from our pathology archives from 1993 to 2006. In addition, tissue microarray sections containing 13 tumors were stained for other NOTCH-family members (NOTCH1, NOTCH3, NOTCH 4), NOTCH2 primary ligand JAGGED1, and γ -secretase component Nicastrin. IHC was performed on Ventana Discovery XT autostainer using standard immunoperoxidase techniques. Appropriate controls were used. The staining intensity was graded as absent, weak, moderate, or strong. The percentage of positively staining cells was graded as none, rare (0-10%), focal (11-50%), or diffuse (>50%). A note was made of the cellular localization.

Results: The 24 hepatoblastomas were from 20 patients ranging in age from 6 days to 68 months (mean 22 months); 16 patients were male and 3 were female. One case had the favorable pure fetal histology; others showed mixed embryonal and fetal histology. Compared to the normal liver, increased NOTCH2 expression (nuclear and cytoplasmic) was seen in 22 of 24 (92%) hepatoblastomas and increased Nicastrin expression (cytoplasmic) was seen in 12 of 13 (92%) tumors. There was no significant staining for NOTCH1/3/4 or JAGGED1 in hepatoblastoma cells. NOTCH3 (strong) and NOTCH1 (weak) stained endothelial cells lining the sinusoids.

Conclusion: Our study shows robust nuclear/cytoplasmic overexpression of NOTCH2 and Nicastrin in hepatoblastomas. The anti-NOTCH2 antibody recognizes an epitope exposed when NOTCH2 is cleaved to produce NICD. Our data indicates that, in hepatoblastoma cells, there is aberrant NOTCH2 activation independent of its interaction with its primary ligand JAGGED1 but likely not independent of cleavage by γ -secretase complex. Interestingly, our only hepatoblastoma with pure fetal histology stained weakly for both NOTCH2 and Nicastrin. Our observations have potential implications with regard to therapeutic targeting of the NOTCH signaling pathway in hepatoblastomas.

9 Transplacental vs Postnatal Regulation of the RAS in Offspring Kidneys by Dietary Salt

Nadezda Koleganova 1, Grzegorz Piecha 2, Eberhard Ritz 2, Gross-Weissmann M.L 1, 1Institute of Pathology, University of Heidelberg, Heidelberg, Germany
2Department of Internal Medicine, University of Heidelberg, Heidelberg, Germany

Background: An adverse intrauterine environment, e.g. high dietary intake of salt, impacts on kidney development (reduced nephronogenesis), lowers the number of glomeruli and causes arterial hypertension later in life. Blockade of the renin-angiotensin system during intrauterine development also reduces nephronogenesis and low number of glomeruli. It was the purpose of the present study to clarify whether high salt intake in pregnant rats affects the renin-angiotensin system and kidney development respectively in the offspring.

Design: Sprague-Dawley rats were fed normal (0.15%), medium (1.3%), or high (8.0%) salt diets during pregnancy and weaning. The offspring were weaned at 4 weeks of age and subsequently switched to normal salt diet. The number of glomeruli and blood pressure were assessed at 12 weeks postnatally (morphometry; Weibel principle). The expression of the components of the renin-angiotensin system (Western blot) in the offspring kidneys was assessed at term and at 1 week of age (end of nephrogenesis).

Results: At postnatal week 1 the number of glomeruli in the offspring of the mothers on high salt diet (10,790±1,219) was significantly lower compared to the other groups (medium 30,421±3,971 and low salt 20,784±2,247). At term the expression of renin in kidneys of the offspring of dams on high salt was reduced, i.e. 69±32% (SD) of that of offspring of dams of the other two groups taken as 100±18%; the respective values for ACE were 64±29% vs. 100±55% and for angiotensin II type I receptor 75±39% vs. 100±35 (all differences <0.005). In contrast at 1 week of age the expression of renin (134±34% vs. 100±25%), ACE (134±33% vs. 100±37%) and angiotensin II type I receptor (131±64% vs. 100±46%) had become significantly higher in the offspring of mothers on high-salt diet compared with the other groups.

Conclusion: We conclude that high maternal salt intake during pregnancy suppresses the renin-angiotensin system in developing kidney of the offspring thus causing reduction of the final number of nephrons; in contrast postnatally in the kidney of the offspring a rebound of the activity of the RAS is observed.

10 Micro CT Imaging of Bladder Outlet Obstruction.

JR Siebert, KJ Smith, IA Glass, T Cox, Departments of Pathology and Pediatrics, Seattle Children's Hospital; Center for Tissue and Cell Sciences, Seattle Children's Research Institute; Univ of Washington; Seattle, WA.

Background: Developments in microtomography now permit the detailed imaging of remarkably small, delicate, and/or complex anatomic specimens. We reported the use

of optical projection tomography earlier (Siebert et al, 2010) and have now extended our studies to include scanning by micro CT. This technique offers high resolution (to 9 microns) scanning of opaque specimens up to 6.8 cm in diameter and 20 cm in length; traditional histologic examination can be performed after scanning when desired. The use of microtomography will expand the morphologic understanding of a number of congenital disorders, including bladder outlet obstruction. Generic bladder outlet obstruction can be identified by prenatal ultrasonography, but more precise anatomical diagnosis will improve counseling and lead to a better understanding of pathogenesis.

Design: To demonstrate this technique, we first present a series of images of the normal urinary bladder and posterior urethra from male fetuses in middle to late gestation. Intact, formalin-fixed autopsy specimens were impregnated with phosphotungstic acid and scanned. Images were then reconstructed using NRecon software (Skyscan, Belgium); virtual sections were rendered for viewing in three dimensions using Bioptics Viewer v2 and Drishti v2.0 software. These were compared to images of specimens altered by suspected posterior urethral valves or severe urethral stenosis/atresia.

Results: The external and internal features of the urinary bladder and posterior urethra are readily visualized in single planes or via three dimensional rendered images. The latter can be rotated at will and sectioned electronically in any plane. Small structures, including the urethral crest, verumontanum, and its components (i.e., prostatic utricle and ejaculatory ducts), can be seen in detail. Control specimens can be compared to those altered by obstructive lesions. In two cases of apparent posterior urethral valves, examination by micro CT revealed a single obstructing diaphragm (type III PUV) rather than individual (type II) valves. One case of atypical urethral stenosis was associated with ectopic rectovesicle fistula and seminal vesicles. In one case of urethral atresia, the bladder wall was fused, without other pathologic changes.

Conclusion: Microtomography is a powerful tool for the study of small specimens. As such, it offers a fresh way to address questions in both clinical and research arenas. Using micro CT, the external and internal features of the intact fetal and neonatal urinary bladder and posterior urethra can be demonstrated, augmenting classic autopsy to facilitate more accurate diagnosis. Had the two cases of type III PUV been examined by traditional dissection, for example, the obstructing diaphragm would have been bisected, yielding an incorrect diagnosis of separate posterior urethral valves. It is possible this artifact has influenced the epidemiologic understanding of bladder outlet obstruction as well.

11 Markers for Distinguishing Components of Male and Female Differentiation in Gonadal Dysgenesis

R Buell-Gutbrod, J Steinmetz, A Montag, K Gwin, Department of Pathology, University of Chicago, Chicago, IL.

Background: In disorders of sex development, gonadal tissue can present as dysgenetic ovarian or testicular tissue, streak gonads, or as undifferentiated gonadal tissue with sex cord cells. The identification of male and female components based on morphology alone can be challenging. FOXL2, a forkhead-winged-helix transcription factor, is one of the first genes expressed during female gonadal development. It is required for granulosa cell differentiation during folliculogenesis and expressed in ovarian stroma. The transcription factor SOX9 is an intermediate downstream target of SRY and is required for testis development by formation and maintenance of (pre-)Sertoli cells. CYP11A1 and STAR are potential markers for Leydig cells (LC) based on the testosterone biosynthetic pathway. INSL3 is produced in adult and fetal LCs and may have a role in the differentiation of the gubernaculum. We hypothesized that the counteracting transcription factors FOXL2 and SOX9 will be useful for distinguishing male and female sex cord stromal components whereas CYP11A1, STAR and INSL3 will be able to identify LCs.

Design: Archival paraffin embedded material of 11 dysgenetic gonads, including ovo-testis, mixed gonadal dysgenesis, streak gonads and Sertoli cell adenoma, were examined by IHC for the expression and localization of FOXL2, SOX9, CYP11A1, STAR and INSL3. Underlying disorders of sex development included Turner, Swyer and androgen insensitivity syndrome. A control group included normal fetal/adult testis (n=10/10) and ovaries (n=5/10).

Results: In ovotestis and mixed gonadal dysgenesis, the sex cord-stromal cells of ovarian type tissue (granulosa cell lineage) revealed strong nuclear staining for FOXL2 and were negative for SOX9. Sex cord-stromal cells in testicular type tissue (Sertoli cell lineage) showed strong nuclear expression of SOX9 and no expression of FOXL2. The Sertoli cell adenoma revealed positivity for SOX9 and negativity for FOXL2. CYP11A1, STAR and INSL3 highlighted the presence of LCs in male gonadal tissue. Streak gonads exhibited nuclear expression of FOXL2 in a pattern similar to normal ovarian stroma and were negative for SOX9. Germ cells were devoid of FOXL2 and SOX9 expression. In the control group, nuclear expression of FOXL2 was observed in immature and mature granulosa cells and in ovarian stroma. SOX9 exhibited nuclear expression in Sertoli cells of fetal and adult testis. Fetal and adult type LCs expressed CYP11A1, STAR and INSL3.

Conclusion: FOXL2 actively suppresses SOX9 throughout adult life via an upstream regulatory element, leading to a mutually exclusive expression of these two transcription factors. Based on the distinct expression of FOXL2 and SOX9, our findings support that these markers are useful to male and female components in dysgenetic gonads. The testosterone biosynthetic pathway markers CYP11A1 and STAR as well as INSL3

are of value to identify LCs in gonadal tissue. Our proposed panel is useful for further classification of lesions within the gonadal dysgenesis group.

12 Eosinophilic/T-cell Chorionic Vasculitis Involves Regulatory T-cells and is Associated with Chronic Villitis

PJ Katzman (1), DA Oble (2), University of Rochester Medical Center, Rochester, NY, USA(1); University of Manitoba, Winnipeg, MB (2).

Background: Eosinophilic/T-cell chorionic vasculitis (ETCV) is a relatively new entity described as the presence of a mixed eosinophilic and T-cell infiltrate that marginates into a single chorionic vessel wall opposite the amnionic surface without accompanying acute maternal and fetal inflammatory responses. Its significance is uncertain. The presence of a mixed T-cell and histiocytic infiltrate can be confirmed using CD3 and CD68 immunostains, respectively. We sought to better characterize this lesion in our population both with respect to other pathological correlates and the T-cell population involved.

Design: Placentas with ETCV were identified from 2004-2010 in the URM surgical pathology laboratory information system searching with the keywords, "placenta" and "eosinophil". Surgical reports and archived slides were reviewed. The presence of ETCV as defined in the literature was recorded as well as any other pathologic diagnosis. Clinical data tabulated included maternal age, gestational age, gravida and para status, fetal and placental weights, and pregnancy complications. After review of pre-existing H+E and immunostains (CD3, CD20, CD68) to confirm the diagnosis of ETCV, an archived block from a subset of these cases was cut and stained with FOXP3 immunostain (Abcam, Cambridge, MA) to identify any regulatory T-cell population in the affected chorionic vessel(s). 8 third trimester placentas were stained with FOXP3 as negative controls. The CD3-positive and FOXP3-positive cells were quantitated by manual counts of representative 40x fields at the site(s) of ETCV and FOXP3:CD3 ratios were calculated for this subset of cases.

Results: Of the 57 cases retrieved, 23 were excluded because eosinophils accompanied an acute fetal inflammatory response without a T-cell population; there were inadequate cells present to confirm ETCV; or orientation of inflammation in the vessel was not diagnostic. Of the 34 remaining cases of ETCV (estimated incidence in our population, 0.39%), 24 were term and 10 preterm, 6 occurred in multiple gestations, 10 were accompanied by chronic villitis (29%), 5 by chronic deciduitis (15%), 6 by a thrombus in the vessel affected by ETCV, 8 by an acute maternal inflammatory response, 5 by an acute fetal inflammatory response, and 3 by an umbilical form of ETCV. The mean FOXP3:CD3 ratio was 0.25 (range 0.05-0.58). Only 1 control placenta contained FOXP3 staining in a focus of previously undiagnosed chronic villitis, but no staining was seen in chorionic vessels.

Conclusion: Chronic villitis was seen in over a quarter of the cases in this population, which has not been

reported in 2 previous studies of ETCV. This finding supports the possibility that ETCV is either a response to infection or an autoimmune process. FOXP3-positive cells represent a significant subpopulation of T cells in ETCV, suggesting that regulatory T-cells play some role in ETCV. Additional cases will be stained for both FOXP3 and CD25, another T regulatory cell marker.

13 Major and Minor Placental Pathology Contribute to Reduced 6 Month Cognitive Scores in Growth Restricted Infants.

LM Ernst, MH Huang, BP Plunkett, K Machut, A Lossia, R Deregner, Prentice Women's Hospital and Children's Memorial Hospital, Northwestern University, Chicago IL. **Background:** Intrauterine growth restriction (IUGR) in the newborn is associated with morbidities such as hypoxic brain injury. An understanding of the prenatal pathologies that contribute to IUGR and its sequelae may help advance our understanding of the neonatal outcomes. **Design:** Patients were enrolled prospectively either prenatally or at birth. Infants with birthweights (BW) <10th percentile comprised the IUGR group, and age-matched control patients had BW between 10-90th percentile. Placentas were collected at birth, and trimmed placental weight recorded. Sections of umbilical cord, membranes, full thickness parenchyma, and any gross lesions were submitted. Placental pathologic findings were classified into two categories: major and minor pathology. Major pathology included maternal vascular underperfusion, fetal thrombotic vasculopathy, massive perivillous fibrin deposition and diffuse chronic villitis. Minor pathology included findings suggestive of maternal vascular underperfusion with a normal placental weight and fetal vascular thrombi/avascular villi that were not extensive enough to be diagnostic of fetal thrombotic vasculopathy. At 6 months corrected age, the Bayley Scales of Infant-Toddler Development were administered, and Cognitive, Language and Motor Composite scores were computed. Infant visual memory was evaluated using the Fagan Test of Infant Intelligence. Statistical analyses were performed and data are presented as the mean \pm SEM.

Results: 83 patients were recruited (48 controls, 35 IUGR). Mean gestational age was similar for the two groups. Mean placental weight was significantly lower in IUGR cases (345.6 ± 21.37 g) than in controls (484.1 ± 17.96 g, $p < 0.0001$). More patients in the IUGR group had major placental pathology (19/35, 50%) than in the control group (7/48, 15%, $p = 0.0001$). 69% of infants with BW <3rd percentile had major pathology, compared to 42% of patients with BW 3-10th percentile, and 15% of control patients ($p = 0.0005$). Mean ferritin levels were significantly lower in IUGR infants (93.0 ± 19.19 ng/ml) than control infants (211.6 ± 50.7 ng/ml, $p = 0.025$). Six month Cognitive Composite scores were significantly lower in infants with major and minor pathologic findings (98.9 ± 2.4) than in the infants without placental pathology (105.8 ± 2.2 , $p = 0.045$). Cognitive Composite scores were similar in infants with major and minor placental pathology. Language and Motor Composite scores did not differ significantly between the groups,

and there was no significant difference in visual memory abilities.

Conclusion: Severity of IUGR was associated with an increasing incidence of major placental pathology, but both major and minor placental pathology were associated with reduced cognitive scores at 6 months of age. Further longitudinal evaluation of these patients is underway, but these data suggest that identification of both major and more minor pathologic lesions is of clinical importance.

14 Prenatal Diagnosis of Trisomy 6 Mosaicism as an Indirect Evidence for Trisomy Rescue Resulting in Paternal UPD6: Genetic Findings and Placental Pathology

Mariana M. Cajaiba, MD1; Selma Witchel, MD2; Suneeta Madan-Khetarpal, MD3; Jaqueline M. Hoover, MS3; Trevor Macpherson, MD1; Urvasi Surti, PhD1, Department of Pathology¹, University of Pittsburgh Medical Center; Departments of Endocrinology² and Medical Genetics³, Children's Hospital of Pittsburgh.

Background: Uniparental disomy (UPD) is defined by the inheritance of both copies of a chromosome pair from one single parent. 21 cases of paternal UPD for chromosome 6 (patUPD6) have been reported, whereas maternal UPD6 is exceedingly rare. The phenotype of patUPD6 results from biallelic expression of the maternally imprinted ZAC and HYMAI genes, and includes transient neonatal diabetes mellitus (TNDM), intra-uterine growth restriction (IUGR), macroglossia and dysmorphic features. Trisomy rescue with random elimination of the normally inherited chromosome has been proposed as a pathogenic mechanism resulting in UPD for other chromosomes. However, neither the association of trisomy 6 and patUPD6, nor the prenatal diagnosis of the latter, have ever been reported.

Design: We describe the clinical, cytogenetic and molecular features of a female infant born at 33 weeks, and also the placental pathology.

Results: The infant was diagnosed with TNDM and IUGR; macroglossia and other minor dysmorphic features were present. The placenta was small for the gestational age and showed dysmorphic villi and a proliferation of abnormally dilated vessels of variable sizes within stem and terminal villi; no features of placental mesenchymal dysplasia were present. Karyotype and UPD studies from cultured amniocytes revealed trisomy 6 mosaic and patUPD6, respectively. Karyotype and FISH studies from the infant's peripheral blood, as well as FISH from skin fibroblasts and buccal smear were negative for trisomy 6. A whole genome chromosome single nucleotide polymorphism (SNP) array performed on the infant's peripheral blood showed complete allele homozygosity for chromosome 6 and normal chromosome dosage, confirming UPD6 in the infant. A karyotype from fresh placental tissue showed trisomy 6 in all examined cells.

Conclusion: We report a unique case in which patUPD6 was detected in a diploid child following a prenatal diagnosis of mosaic trisomy 6, with post-natal confirmation of trisomy 6 restricted to the placenta, thus

suggesting trisomy rescue. Although prenatal studies suggested fetal trisomy 6 in our case, it was subsequently excluded. Two possibilities could explain our findings: 1) contamination from extra-embryonic trisomic cells, suggesting confined placental mosaicism; or 2) a cell line-restricted mosaicism in some fetal tissues. As in other prenatally diagnosed mosaic trisomies, accurate prediction of the fetal karyotype poses a challenge to their management, and our observations reinforce the importance of UPD screening when indicated. The placental findings were also remarkable, and have not been described within the spectrum of UPD6 or trisomy 6.

15 Premature Infants Utilize Accelerated Expression of Pulmonary Serotonin Transporter (SERT) for Pulmonary Vascular Adaptation and Survival

EC Castro, C Galambos, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA.

Background: Recent evidence suggests that the survival of less than 28 week old premature babies can be explained by their biological adaptability/plasticity. Recent work from our laboratory has identified SERT as a key regulator of pulmonary vascular adaptation at birth and that its absence contributes to the pathomechanism of alveolar capillary dysplasia. We have also shown that normally SERT is expressed by pulmonary endothelial cells starting at 30 weeks in utero and continues to be expressed strongly throughout life. We hypothesized that in infants who survive premature birth SERT will become active earlier indicating an induction and activation of a biological adaptation process in very premature infants.

Design: Six cases of premature babies with a median survival of 30 days post-delivery were selected. Lung sections were stained with antibodies to SERT.

Results: The median gestational age was 29 weeks (24-35) with a median survival of 30 days (1-56). The causes of death were necrotizing enterocolitis (n=3), placental complications (n=2) and infection (n=1). All infants received surfactant after delivery and corticosteroids prior to onset of labor. SERT expression was strong and diffuse in 3 cases and 2 cases showed focal SERT staining. In the extremely premature infant (24 weeks) SERT staining was absent.

Conclusion: There is onset of accelerated pulmonary endothelial cell SERT activity in premature babies. This is probably required for survival considering the crucial role SERT plays in the process of pulmonary vascular adaptation at birth. If the results hold up in a larger series, the observation of accelerated SERT expression supports the concept that there is biological adaptability in premature infants that facilitates pulmonary vascular adaptation and survival.

16 Biogenic Amine Transport System Immunohistochemical Computational Analyses Characterization as a Guide for Diagnoses and Management of Hyperserotonemic Disorders During Pregnancy.

Castro, ECC, Parks, Tony W and Galambos, C, Children's Hospital of Pittsburgh and Magee Women's Hospital of the University of Pittsburgh Medical Center

Background: The intrauterine environment is characterized by high catecholamine secretion and clearance. This clearance is mediated in large measure by placental transport proteins. Alterations in the function or capacity of this transport system are likely to have significant effects on the developing fetus. Pregnant illicit drug abusers (especially cocaine, CO), selective serotonin reuptake inhibitor (SSRIs) users, and a number of mothers with pregnancy induced hypertension (PIH) have all elevated serum serotonin levels thus the term hyperserotonemic disorders (HD). Serotonin uptake transporter (SERT) of cytotrophoblasts has been shown to control serotonin level in the placenta. We hypothesized that altered SERT expression may contribute to pregnancy related hyperserotonemic disorders.

Design: 16 normal placentas representing mid and term gestational age were used to determine normal SERT expression pattern. In addition 10 CO, 11 SSRIs and 20 PIH placentas were collected. Slides were stained with SERT antibody. Quantitative morphometric immunostain analysis was done by using Nuance VIS-FL Multispectral Imaging System. The percentage of positive stained area with cells showing DAB positive membrane/cytoplasmic positivity/100 high power fields was acquired and compared between the two trimesters of gestation (control group) and PIH, SSRI and CO placentas. For the statistical analysis, Mann-Whitney, Kruskal-Wallis, and analysis of variance tests were performed.

Results: Predominantly membranous SERT staining was seen in the villous/extra villous cytotrophoblasts and the expression showed a slight increase towards the end of the gestation. PIH placentas (4.2%) had similar SERT expression to that of controls (4.1%), but significantly higher when compared to that of CO (2.3%) and SSRIs placentas (2.1%). When the groups were matched for gestational age with the controls (3.7%) the CO placentas (3.5%) and the SSRIs (2.4%) placentas showed significantly decreased SERT expression (p=0.001). The lowest expression was observed in the SSRIs group when compared with controls (p<0.05).

Conclusion: As far as we know there are no published studies which were designed to compare the immunohistochemical profile of these biogenic amines and their transporters in placentas from fetus exposed to cocaine, SSRIs or hypertension during pregnancy. SERT expression is decreased in cocaine and anti-depressant users which can be the primary cause of the elevation in the serotonin levels observed in these patients. SERT expression was not altered in PIH. It is possible that other catabolic component of serotonin metabolism (i.e monoamine oxidase) is altered, which might explain the high serotonin levels in these select patients.

17 Omental Fibromyxoid Tumors (OFT) and Inflammatory Myofibroblastic Tumors (IMT): A Diagnostic Signature and Biological Differences by High Throughput MicroRNA Expression Profiles

ST Sredni and PM Chou, Children's Memorial Hospital and Children's Memorial Research Center, Northwestern University/Feinberg School of Medicine, Chicago, IL.

Background: Omental fibromyxoid tumor, originally described by Gonzalez-Crussi as omental-mesenteric myxoid hamartoma, is considered part of the morphologic spectrum of IMT. Recently we have found subtle differences in their clinical, histological and immunohistochemical profiles. MicroRNAs (miRNAs) are short regulatory RNAs that negatively modulate protein expression at a post-transcriptional level. miRNAs can regulate several messenger RNAs simultaneously by mechanisms such as incomplete base pairing and post-transcriptional gene silencing. Their deregulation has been correlated with many biological processes and diseases.

Design: The aim of this study was to investigate the miRNA expression profile of OFT and IMT to further delineate these tumors. To achieve this goal, four frozen primary tumor samples (2 OFT and 2 IMTs) were compared. Total RNA was extracted using miRCURY RNA Isolation Kit (Exiqon Vedbaek, Denmark). Array experiments were conducted as double-channel Hy3/Hy5 experiments in triplicates on Exiqon's miRCURY LNA microRNA Array, v.11.0. Controls: reference RNA samples were mixed pair-wise and hybridized simultaneously. After normalization fold change (FC) and p-values were calculated and utilized for selection of the miRNAs that are significantly differentially expressed. Hierarchical clustering was performed using Cluster software and heat maps using TreeView (Eisen, 1998). Potential targets were identified using miRDB bioinformatics tool (<http://mirdb.org/miRDB>). Molecular functions of the potential protein-coding target genes were assessed using Ingenuity Pathways Analysis (IPA) software v8.7.

Results: Our result showed 8 differentially expressed miRNAs. In OFTs, four were up-regulated-miR-431 (FC 1.6; p 0.04), miR-487a (FC 1.4; p 0.04), miR-637 (FC 2.7 p 0.06) and miR-664 (FC 2.0; p 0.07); and four down regulated miR-9 (FC 2.6; p 0.02), miR-299-5p (FC 3.2, p 0.02), miR-606 (FC 1.8; p 0.01) and miR-1298 (FC 1.9; p 0.04)]. Using miRDB, 1,005 potential target genes were identified. Targets with score ≥ 80 were selected and the list of 171 genes was analyzed with IPA. The result showed that the top function genes represented by this list are those involved in DNA replication and repair (p 0.027), cellular development (p 0.035), embryonic development (p 0.035) and cell death (0.037).

Conclusion: OFTs are benign tumors that occur in the mesentery of young children. In contrast, IMTs occur anywhere in the body and has varied biologic behavior. As such, it is important that these two tumors should be differentiated. This preliminary study suggests that there are different miRNAs expression profile between OFTs and IMTs. It further supports our previous finding of different immunohistological profile of these two tumor types and may give clues to their biological differences.

Further studies in larger cohorts are necessary to validate these findings.

18 Cervical Embryonal Rhabdomyosarcoma. A Unique Pathologic Type Of ERMS and Its Associations.

Louis P. Dehner, Jason Jarzembowski and D. Ashley Hill, Lauren v. Ackerman Laboratory of Surgical Pathology, Barnes-Jewish and St. Louis Children's Hospitals, Washington University Medical Center, St. Louis; Department of Pathology and Laboratory Medicine, Children's Hospital of Wisconsin, Medical College of Wisconsin, Milwaukee, WI; Division of Pathology, Children's National Medical Center, Washington, DC.

Background: Embryonal rhabdomyosarcoma (ERMS) of the uterine cervix is differentiated from vaginal and other lower genitourinary tract ERMSs by its presentation in older children and adolescents, its cartilaginous nodules and its favorable outcome in most cases.

Design: The surgical pathology files of our institutions were searched for all examples of cervical ERMS presenting in the first two decades of life. A total of 12 cases were identified. Hematoxylin and eosin-stained sections were examined to confirm the diagnosis and immunohistochemical stains were performed in those cases with available blocks.

Results: The age range at diagnosis between 9 mo and 19 yr (mean 13 yr, median, 13 yr), vaginal bleeding of several days to weeks duration and a polypoid mass measuring 1.5 - 3 cm at the cervical os characterized the 12 cases. Polypectomy was the initial surgical procedure for all patients. All but one tumor had similar histological features; endocervical glands surrounded by condensed, concentric mantles of primitive small cells, with or without apparent rhabdomyoblastic differentiation as highlighted by desmin and myogenin. Nodules of immature, cellular cartilage were present in 5 (40%) of 12 cases. The epithelial structures and their relationship to ERMS and chondroid nodules resembled the architecture of type I (cystic) pleuropulmonary blastoma (PPB). The one variant case in a 9 mo contained immature epithelial structures with neuron-specific enolase positivity rather than cartilage in the stroma. One 13 yr also had an ovarian Sertoli-Leydig cell tumor (SLCT), one 12 yr had a past history of a PPB at age 3 yr and another 9 yr had an involuted PPB. SLCT has been found in association with the PPB-tumor predisposition syndrome.

Conclusion: Cervical ERMS has unique morphologic features which have an uncanny resemblance to cystic PPB. These tumors occur in older children and adolescents and usually have a favorable outcome if anaplasia is absent. The youngest child in this series had a tumor with neuroectodermal-like features. PPBs were found in 2 cases and SLCT in another, raising the possibility of a relationship between cervical ERMS and PPB.

19 Loss Of Immunohistochemical Expression of γ -catenin is a Sensitive Marker of Metastatic Ewing Sarcoma

J Han, S Khokhar, CF Timmons, D Rakheja, UT Southwestern Medical Center, Dallas, TX; Children's Medical Center, Dallas, TX.

Background: Ewing sarcoma family of tumors (EWS) represents the second most common malignant bone tumors in children. At initial presentation, 25% of the patients have metastatic disease and a 6-year survival of 28%. Of the patients with initially localized disease, 35% develop recurrence with a 5-year post-relapse survival of 14%. No currently available clinical or pathologic parameters accurately predict metastasis/recurrence potential of EWS. The aim of this study is to identify protein biomarkers that show differential expression in localized versus metastatic disease with the goal of predicting metastasis/recurrence in initially localized EWS.

Design: We performed immunohistochemistry (IHC) on formalin-fixed/paraffin-embedded tissue microarray sections containing 22 EWS retrieved from our pathology archives from 1998 to 2008. IHC was performed on Ventana Discovery XT (Tucson, AZ) autostainer using standard immunoperoxidase techniques. Appropriate controls were used. IHC was performed for the following metastasis-related protein biomarkers involved in cell adhesion/migration or signaling: NCAM, Vinculin, Robo 1, MMP9, TIMP2, TGF beta receptor I, TGF beta receptor II, IGF1 receptor, Axl, integrin beta 1, γ -catenin, OB cadherin, and MUC16 (Abcam); PTPRE, JAM3, and Nicastrin (Sigma Aldrich); CD70 and PTPRM (Life Span); Numb and Ral B (Santa Cruz).

Results: The patients in our cohort ranged in age from 18 months to 33 years (mean 13.1 years); 12 were male and 10 were female. 14 patients presented with localized disease without subsequent recurrence or metastasis (mean follow up 4.9 years, range 11 months to 10 years). 8 patients presented with metastatic disease (6) or developed metastasis/recurrence during follow-up (2). Diffuse membranous γ -catenin positivity was seen in 7 of 14 (50%) localized EWS, while no tumor (0/8) with metastatic disease stained for γ -catenin. No significant differential expression between localized and metastatic disease was noted for the other markers.

Conclusion: γ -catenin, a component of the cadherin-catenin complex, functions in cell adhesion and Wnt signaling. While γ -catenin is a transcription factor and an oncogene, it also acts as a metastasis/invasion suppressor. The loss of γ -catenin may cause reduced intercellular adhesion and increased cell motility, contributing to the invasive potential of tumor cells. Reduced expression γ -catenin has been associated with adverse prognosis in bladder, breast, oral, and lung carcinomas and neuroblastoma. Our results show that loss of γ -catenin immunoreactivity is a sensitive although not a specific marker of metastatic EWS. However, EWS relapse may occur late (up to 18 years in one study). Therefore, larger studies with longer follow-up are needed to fully evaluate the potential of γ -catenin as a candidate prognostic marker in EWS.

20 Acute Megakaryoblastic Leukemia (AMkL): The COG AML0531 Experience

KM Chisholm¹, S Kahwash², BA Hirsch³, SC Raimondi⁴, TA Alonzo⁵, RB Gerbing⁶, R Aplenc⁷, L Sung⁸, L Winter⁹, K Glick¹⁰, P Byron¹¹, L Burden⁶, T Wallas⁶, SM Davies¹², FO Smith¹², S Meshinchi¹³, AS Gamis¹⁴, and A Heerema-McKenney¹, ¹Dept. of Pathology, Stanford University Medical Center, Stanford, CA; ²Nationwide Children's Hospital, Columbus, OH; ³Dept. of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN; ⁴Department of Pathology, St. Jude Children's Research Hospital, Memphis, TN; ⁵Keck School of Med. Dept. of Preventive Medicine, Univ. of Southern California, Arcadia, CA; ⁶Children's Oncology Group, Arcadia, CA; ⁷Children's Hospital of Philadelphia, Philadelphia, PA; ⁸Dept. of Pediatrics, Div. of Hem./Onc., The Hospital for Sick Children, Toronto, ON, Canada; ⁹Seattle Children's Hospital, Seattle, WA; ¹⁰Maine Children's Cancer Program, Scarborough, ME; ¹¹British Columbia Children's Hospital, Vancouver, BC, Canada; ¹²Div. of Hematology/Oncology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; ¹³Clinical Research, Fred Hutchinson Cancer Rsrch. Ctr., Seattle, WA; ¹⁴Children's Mercy Hospitals & Clinics, Kansas City, MO.

Background: AMkL is a rare subtype of acute myeloid leukemia (AML) more common in children. We report features of non-Down syndrome AMkL cases at central review for the COG trial AML0531.

Design: All cases with an institutional or central review diagnosis of AMkL were identified from the AML0531 database. The patient age at diagnosis, karyotype, immunophenotype from submitting institution and results of any stains performed at central review were retrieved. Slides were reviewed for obliterating fibrosis and the presence of multilineage dysplasia (MLD).

Results: Of the 63 cases submitted with a diagnosis of FAB M7, 47 cases were confirmed (75%), accounting for 5% of centrally reviewed cases. From the 47, the mean age was 2.3 years, and the median was 1.45 years. All but 1 of the 47 had central cytogenetics review, and in general the cases had complex karyotypes. Using the 2000 WHO classification, there were 43 AMkL not otherwise specified (NOS), 2 AML with 11q23 abnormality, and 2 AML with MLD. Using the 2008 WHO classification, there were 15 AMkL, NOS, 24 AML-with myelodysplasia related changes (AML-MRC), 7 AML with t(1;22) and 1 AML with t(9;11). All cases expressed CD61 by flow cytometry when assessed. Common AML antigens were inconsistently expressed: CD13 (38%), CD33 (68%), CD34 (38%), CD117 (38%). "Aberrant" CD4 (32%) and CD7 (36%) expression were seen, with 14/24 cases of AML-MRC expressing CD7. Obliterating fibrosis was seen in several subcategories, but most frequent in AML with t(1;22). MLD was rarely identified.

Conclusion: In this largest series to date, AMkL displays a morphologic, immunophenotypic and cytogenetic heterogeneity previously not appreciated. The 2008 WHO classification stratifies the entity by cytogenetic subgroups, with the largest being AML-MRC

by virtue of a myelodysplasia related karyotype. We await outcome data from AML0531 to see if these subcategories have prognostic significance.

21 Acute Erythroleukemia (AEL): The COG AML0531 Experience

KM Chisholm¹, S Kahwash², SC Raimondi³, BA Hirsch⁴, TA Alonzo⁵, RB Gerbing⁶, R Aplenc⁷, L Sung⁸, L Winter⁹, K Glick¹⁰, P Byron¹¹, L Burden⁶, T Wallas⁶, SM Davies¹², FO Smith¹², S Meshinchi¹³, AS Gamis¹⁴, and A Heerema-McKenney¹, ¹Dept. of Pathology, Stanford University Medical Center, Stanford, CA; ²Nationwide Children's Hospital, Columbus, OH; ³Department of Pathology, St. Jude Children's Research Hospital, Memphis, TN; ⁴Dept. of Laboratory Medicine and Pathology, University of Minnesota Medical School, Minneapolis, MN; ⁵Keck School of Med. Dept. of Preventive Medicine, Univ. of Southern California, Arcadia, CA; ⁶Children's Oncology Group, Arcadia, CA; ⁷Children's Hospital of Philadelphia, Philadelphia, PA; ⁸Dept. of Pediatrics, Div. of Hem./Onc., The Hospital for Sick Children, Toronto, ON, Canada; ⁹Seattle Children's Hospital, Seattle, WA; ¹⁰Maine Children's Cancer Program, Scarborough, ME; ¹¹British Columbia Children's Hospital, Vancouver, BC, Canada; ¹²Div. of Hematology/Oncology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH; ¹³Clinical Research, Fred Hutchinson Cancer Rsrch. Ctr., Seattle, WA; ¹⁴Children's Mercy Hospitals & Clinics, Kansas City, MO.

Background: AEL is a rare subtype of acute myeloid leukemia (AML) primarily affecting older adults, similar to the FAB M6 designation. The WHO recognizes two types: erythroid/myeloid and pure erythroid leukemia. We report features of non-Down syndrome AEL cases at central review for the COG trial AML0531.

Design: All cases with an institutional or central review diagnosis of FAB M6 morphology were identified from the AML0531 database. The patient age at diagnosis, karyotype, immunophenotype from submitting institution and results of any stains performed at central review were retrieved. Slides were re-reviewed for the presence of multilineage dysplasia.

Results: Of the 18 cases submitted as FAB M6, 14 cases were confirmed (1.6% of centrally reviewed cases). All had cytogenetics confirmed at central cytogenetics review. 5 had complex karyotypes, occurring in the 5 youngest patients in the group, less than 3 years of age. None had $-5/\text{del}(5q)$ and $-7/\text{del}(7q)$. Three cases were pure erythroid leukemia AEL, also restricted to patients less than 3 years of age. 10 cases were erythroid/myeloid AEL. 1 case was AML with multilineage dysplasia. Some authors question whether the erythroid/myeloid cases are better considered MDS. 9 cases could be considered high-grade MDS if the blasts are counted from all nucleated cells as in other types of AML. Of these 9 cases, 7 had multilineage dysplasia, and 3 had an MDS-associated karyotype. Blasts of AEL-pure erythroid leukemia expressed CD4 (2/3), glycophorin A (2/2), CD61 (2/2), and CD33 (1/3), but not CD13 or CD34.

Conclusion: AEL is a rare subtype of AML in children. Pure erythroid leukemia is very uncommon and appears only in the youngest children of our group. In addition, the youngest patients have the most complex karyotypes. Many features overlap with high-grade MDS, the controversy regarding optimal classification continues.

22 STAR and CYP11A1 Expression in Gonadoblastomas

R Buell-Gutbrod, J Steinmetz, A Montag, K Gwin, University of Chicago, Department of Pathology

Background: Gonadoblastomas (GB) are rare mixed germ cell-sex cord stromal tumors that almost exclusively arise in dysgenetic gonads containing the testis-specific protein Y-encoded gene. They are composed of immature germ cells, sex cord-stromal derivatives and may contain clusters of luteinized or Leydig-like cells devoid of Reinke crystalloids. Clinically, occasional secretion of steroid hormones has been described. The steroidogenic acute regulatory protein (STAR) mediates cholesterol transport from the outer mitochondrial membrane to the inner mitochondrial membrane. The mitochondrial enzyme Cytochrome P450, family 11, subfamily A, polypeptide 1 (CYP11A1) catalyzes the conversion of cholesterol to pregnenolone, which is the first and rate-limiting step in the biosynthetic steroid pathway. The aim of our study was to further characterize the expression and localization of the two steroidogenic pathway markers STAR and CYP11A1 in GBs.

Design: Archival paraffin embedded material of 5 GBs, 3 of which showed overgrowth by dysgerminoma, and two arising in streak gonads, were examined by IHC for expression and localization of STAR and CYP11A1. Staining intensity was scored on a scale of 0-3. Normal testicular tissue was used as control. Underlying disorders of sex development included Turner, Swyer and Frasier syndromes. All patients were phenotypically female.

Results: All 5 GBs revealed cytoplasmic staining of the luteinized / Leydig-like cells for STAR (3+=40%, 2+=40%, 1+=20%) and CYP11A1 (3+=100%, 2+=0%, 1+=0%). Areas of transition between GB and dysgerminoma revealed gradual reduction of STAR/CYP11A1 expressing luteinized/Leydig-like cells. Germ cells, sex cord derivatives and gonadal stroma were devoid of STAR and CYP11A1 expression. In the control group, Leydig cells revealed cytoplasmic staining for STAR and CYP11A1 (2-3+), whereas Sertoli cells and germ cells were negative for both markers.

Conclusion: Previous studies, mostly based on radioimmunoassays, demonstrated steroid hormone production in dysgenetic gonads including GBs. Both the luteinized / Leydig-like cells as well as the stroma of streak gonads were proposed as source of steroid hormone production. Based on consistent STAR and CYP11A1 expression in the luteinized / Leydig-like of GBs, our results support participation of these cells in steroid hormone production. No evidence of steroid hormone expression in streak gonadal tissue was observed in this study. In addition, STAR/CYP11A1 appear to be useful markers for evaluation of overgrowth

by dysgerminoma and to identify the transition zone and nests of "dysgerminoma in situ" in which STAR/CYP11A1 demonstrate gradual loss of luteinized/Leydig-like cells to complete absence, respectively.

23 MTORC1 Signaling Pathway is Differentially Active in Yolk Sac Tumors and Seminomas

D Rakheja, P Kapur, S Khokhar, N Fustino, Y Lotan, J Brugarolas, JF Amatruda, UT Southwestern Medical Center and Children's Medical Center, Dallas, TX.

Background: Germ cell tumors (GCTs) are typically classified into the seminomas (dysgerminomas/germinomas) and non-seminomatous germ cell tumors (NSGCTs) such as yolk sac tumors. The former are radiosensitive while latter are treated with chemotherapy that typically consists of bleomycin, etoposide, and cisplatin, which have significant short-term and long-term toxicities. Despite chemotherapy, the 5-year overall survival for poor-risk group patients with NSGCTs is 48%, indicating a need for better therapeutic options. The mammalian target of rapamycin (MTOR) is a serine/threonine kinase that controls key cellular processes such as survival and proliferation in response to nutrients, growth factors, cellular energy, and stress. Rapamycin-sensitive MTOR complex 1 (MTORC1) upregulates HIF-1 α expression, while HIF-1 α effector REDD1 downregulates MTORC1. These pathways, which are known therapeutic targets, have not been studied in germ cell tumors.

Design: We performed immunohistochemistry (IHC) on formalin-fixed/paraffin-embedded tissue microarray sections containing 14 yolk sac tumors and 9 seminomas. IHC was performed on Dako (Carpinteria, CA) or Ventana (Tucson, AZ) autostainers using standard immunoperoxidase techniques. The activation of the MTORC1 pathway was investigated with antibodies to phospho-(Ser2448)-MTOR (pMTOR, Cell Signaling), phospho-(Ser235/236)-S6 ribosomal protein (pS6RP, Cell Signaling), and cyclin D1 (NeoMarkers). The HIF-1 α pathway was queried with antibodies to REDD1 (Bethyl) and GLUT1 (Lab Vision). Appropriate controls were used. Staining intensity was scored as 0 (absent), 1 (weak), 2 (moderate), or 3 (strong). The percentage of positively staining cells was scored as 0 (none), 1 (<10%), 2 (10-50%), or 3 (>50%). A combined IHC score was calculated as the product of the above scores.

Results: Of the 14 yolk sac tumors, 14 (100%) stained for pMTOR (mean IHC score 4.6), 12 (86%) stained for pS6RP (mean IHC score 4.6), 13 (93%) stained for cyclin D1 (mean IHC score 2), 14 (100%) stained for GLUT1 (mean IHC score 6.1), and 14 (100%) stained for REDD1 (mean IHC score 4.6). Of the 9 seminomas, 1 (11%) stained for pMTOR (mean IHC score 0.1), 2 (22%) stained for pS6RP (mean IHC score 0.1), 3 (33%) stained for cyclin D1 (mean IHC score 0.1), 8 (89%) stained for GLUT1 (mean IHC score 4.3), and 9 (100%) stained for REDD1 (mean IHC score 8.5). Two-tailed t-test showed significant differences between the two sets of tumors for the expression of pMTOR (P=0.0000), pS6RP

(P=0.0003), cyclin D1 (p=0.0033), and REDD1 (P=0.0000).

Conclusion: Our study shows that MTORC1 signaling is strongly active in most yolk sac tumors but not in seminomas. Therefore, MTORC1 inhibitors might be of therapeutic value in yolk sac tumors. It is known that REDD1, under induction by PLZF, inhibits MTORC1 in spermatogonial progenitor cells. Our data suggests that REDD1-induced inhibition of MTORC1 might play a role in the maintenance of the undifferentiated phenotype of seminomas.

24 Is Fluorescence In-Situ Hybridization (FISH) Testing Useful in the Differential Diagnosis of Pediatric Melanomas?

JN Punial, V Mehta, C Rosales, WL Wang, AJ Lazar, VG Prieto, DH Lopez-Terrada, 1Division of Molecular Pathology, Department of Pathology, Texas Children's Hospital/Baylor College of Medicine and 2Department of Pathology, MD Anderson Cancer Center, Houston Texas.

Background: The gold standard for differentiating melanoma from benign melanocytic nevi is histopathologic examination with emphasis on cytologic and architectural atypia, and presence of dermal mitotic figures. However, some lesions show overlapping features, making diagnosis based on histology difficult in a subset of cases. Recent studies have shown that fluorescence in-situ hybridization (FISH) testing using a panel of four probes targeting 6p25 (RREB1), centromere 6, 6q23 (MYB) and 11q13 (Cyclin D1), can be a useful additional diagnostic tool for differentiating melanoma from benign nevi in adults. Children can have melanoma and the purpose of our study was to examine the diagnostic utility of this assay in pediatric pigmented lesions where diagnosis is further complicated by the rarity of its occurrence.

Design: Archived pediatric melanoma cases were identified and reviewed. 11 pediatric melanoma specimens from 9 patients of ages ranging from 2 to 17 years were identified. 2 of these patients had a corresponding metastatic focus for comparison analysis with the primary tumor. Of the tumors from the 9 patients, 2 had "spitzoid" morphology, 1 was a small cell variant and 1 was melanoma in-situ. 3 patients had only the metastatic focus for evaluation. 1 patient was excluded as the tumor showed abundant melanin pigment, masking the FISH signals. Using three probes, centromere 6 (spectrum aqua), 6q23 (spectrum gold) and 11q13 (spectrum green), FISH analysis was performed to assess for loss or gain of the specific loci enumerated by these probes. Results were compared to that of the published studies in adult melanomas. The control arm of our study comprised of 5 benign nevi.

Results: All benign nevi showed normal copy numbers of FISH signals. Among the 10 melanoma specimens studied, 60% showed significant gain or loss of the specific loci illustrated by the three FISH probes. 1 case had matted tumor cells making individual cell analysis difficult, but nonetheless, had rare individual cells with abnormal FISH signals. The small cell variant melanoma

did not show any significant loss/gain. 4 cases with unequivocal malignant morphology did not show any significant loss/gain of signals.

Conclusion: FISH analysis is a useful additional tool for distinguishing melanomas from benign nevi in pediatric population. Using a panel of 3 probes mapping previously published loci, 6p23, cep6 and 11q13, we were able to detect abnormality in 60% of the melanomas tested, while none were detected in the benign lesions. Our laboratory is currently evaluating a fourth locus (6p25- RREB1) that, when added to our current panel may increase the sensitivity of this multiplex FISH assay for the diagnosis of pediatric melanoma cases.

25 Beta Adrenergic Receptor Expression in Pediatric Vascular Lesions

KM Chisholm (1), KW Chang (2), MT Truong (2), S Kwok (1), RB West (1), and A Heerema-McKenney (1), (1) Department of Pathology, Stanford, and (2) Department of Otolaryngology, Stanford University School of Medicine, Stanford CA.

Background: Propranolol has recently emerged as an effective therapy for infantile hemangioma (IH) causing regression. The B-adrenergic receptor antagonist is thought to cause vasoconstriction by its effect on nitric oxide, block angiogenesis by its effect on vascular endothelial growth factor (VEGF), and induce apoptosis. We recently reported immunohistochemical staining of beta-2 adrenergic receptors (B2-AR) and their phosphorylated form (B2-ARP) in a case of IH responding to propranolol treatment (*J.Pediatr.* 156:335; 2010). We now explore expression of B-adrenergic receptors on a variety of pediatric vascular lesions using a tissue microarray (TMA).

Design: The TMA contains 45 lesions each represented by two cores: IH (5), epithelioid hemangioma (4), kaposiform hemangioendothelioma (4), intramuscular VM (2), bone hemangioma (2), lymphatic vascular malformation (VM) (7), venous VM (7), glomus tumor (5) pyogenic granuloma (PG) (2), Masson's (2), and one case each of angiomas, VM (mixed small and large vessel), VM NOS, bone hemangioendothelioma, and hobnail hemangioendothelioma. The TMA was immunostained for B2-AR, B2-ARP, and beta-3 adrenergic receptor (B3-AR), and the results scored for the intensity of endothelial cell expression as negative, weak positive, or strong positive. The higher of the two scores was used for analysis.

Results: Strong expression of B2-AR was present in all cases of IH, bone hemangioma, bone hemangioendothelioma, intramuscular VM, VM (mixed small and large vessel), and VM, NOS. Strong expression of B2-ARP was present in all cases of epithelioid hemangioma, VM (mixed small and large vessel), VM NOS, and Masson's, and in most infantile hemangiomas, kaposiform hemangioendotheliomas, and venous VM. Strong expression of B3-AR was present in all cases of IH, bone hemangioma, intramuscular VM, VM (mixed small and large vessel), and Masson's, and the majority of epithelioid hemangiomas. There was absent to weak expression of all three antigens in glomus tumor, hobnail

hemangioendothelioma, angiomas, lymphatic VM, and PG. Strong expression of B2-AR and its phosphorylated form B2-ARP were not necessarily congruent, but weak expression of the counterpart was usually present. All phases of IH had strong expression of all three receptor antigens, with exception of only weak expression of B2-ARP in the proliferative phase IH.

Conclusion: This is the first study to report B-adrenergic receptor expression in these lesions. While immunohistochemical expression of the receptors does not necessarily indicate that similar pathways of responsiveness to beta-blockade are present, it does raise the possibility that beta-blockade could potentially affect apoptosis and decreased responsiveness to VEGF. Additional study is warranted as therapeutic options are limited for some patients with these lesions.

26 Congenital Melanocytic Nevi: Revisiting The Concept Of "Maturation With Depth"

A Davis, A Heider, D Basu, A Rebbaa, M Reyes-Mugica, Department of Pathology, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA.

Background: Giant congenital melanocytic nevi (GCMN) are hamartomatous, tumoral malformations composed of initially modified melanocytes, i.e. nevus cells (NVCs), derived from neural crest cells (NCs). Melanocytes reach the skin through a complex process of migration through pathways similar to those of other elements such as skin nerves. GCMN are present at birth or shortly thereafter, and their NVCs had no time to be exposed to the usual stressors (i.e. UV light) causing adult-age nevi, making their pathogenesis necessarily different to that of acquired nevi. Furthermore, peeling of nevi is usually followed by clinical repigmentation from their base. However, the traditional histological criteria to assess them are based on approaches designed to evaluate acquired nevi. The aim of our study was to explore the expression of markers that may indicate the progressive maturation of NVCs within GCMN.

Design: A panel of immunohistochemical markers present in NCs and/or NVCs was applied to sections from blocks selected of 23 patients with GCMN. The markers included CD56, Tyrosinase, C-KIT, MART1, and HMB45; Microphthalmia inhibitor factor (MIFT), important for nevomelanocyte and melanoma cell survival avoiding apoptosis, was also assessed. We evaluated staining in regards to four levels of deepness: dermo-epidermal junction, papillary dermis, reticular dermis, and subcutaneous tissue. An alkaline phosphatase reaction with red chromogen was used to avoid confusing melanin with diaminobenzidine. A correlation between cell morphology and immunoreactivity was established.

Results: Earlier markers, such as CD56, tend to stain the deeper portions of GCMN (subcutaneous tissue and reticular dermis), and tend to diminish or disappear in superficial layers (papillary dermis and dermo-epidermal junction). More mature markers, such as HMB45 and Tyrosinase tend to be stronger in superficial layers compared with deeper portions. MIFT stains equally all nevus layers.

Conclusion: Although "maturation with depth" is a traditionally held concept, theoretical embryology, clinical observation of repigmentation after nevus peeling, and immunohistochemistry of maturation markers, may indicate a need to extend our inquiry and possibly to reconsider our interpretation of the maturation process in congenital nevi. Traditional concepts derived from analysis of acquired, adult nevocytic lesions, may not be applicable to congenital, developmental melanocytic proliferations.

27 Epithelial Mesenchymal Transition (EMT) is an Earlier Cellular Response to Stress than Senescence: Possible Implication in the Formation of Pre-malignant Tumors

A Rebbaa, D Basu, and M Reyes-Mugica, Department of Pathology, Children's Hospital of Pittsburgh of UPMC, Pittsburgh, PA.

Background: EMT is a reversible differentiation program by which epithelial cells detach from each other and acquire migratory/invasive capabilities. It was first characterized in early developmental processes such as gastrulation and formation of the neural crest. EMT has been also implicated in pathological processes such as fibrosis and cancer. Current evidence supports a key role of EMT in metastasis. In this context, it is noteworthy that EMT and senescence, known as a barrier to cancer, are mutually exclusive. However, the relative chronological occurrence of EMT and senescence is not yet defined. Here we set out to determine which one of these two processes occurs first in response to stimuli, and how they may regulate each other, aiming to better understand the development of giant congenital nevi and associated malignant tumors.

Design: Embryonic stem cells (epithelial), their derivatives (mesenchymal), as well as metastatic and non-metastatic melanoma cell lines (WM 115 and WM266) were subjected to treatment with a known EMT inducer, TGF- β , or senescence inducers H₂O₂ and doxorubicin. The effects of these treatments on cellular proliferation and expression of Zeb1, Twist1, p21, p16 and SA- β Gal were determined. To address the regulatory influence of senescence and EMT between one another, the 293 human embryonic kidney cells were engineered to over-express EMT genes: GSK S9A, GSK-K85A, Zeb1 and Zeb2. Expression of the senescence-associated genes p16 and p21/WAF1 was compared between transfected and non-transfected cells. Inversely, the cells were transfected with the genes coding for p21/WAF1 or p16, and the effects on expression of EMT genes were measured.

Results: Our findings indicate that regardless of the cells or stimuli used, expression of EMT markers occurred before the onset of senescence. Western blot data showed that expression of EMT genes increased in a dose dependent manner while cells are still in their proliferating phase. Higher drug concentrations, which inhibit proliferation, led to a decline in EMT gene expression with a concomitant increase in p21/WAF1 levels and SA- β Gal staining, and to irreversible growth arrest. In regard to the relationship between senescence

and EMT, preliminary data indicate that neither proliferation nor expression of senescence genes was significantly affected in 293 cells engineered to over-express EMT genes. Additional experiments are ongoing to determine whether the reverse effect is valid.

Conclusion: Our findings reveal that EMT occurs earlier than senescence in response to stress. This temporal relationship suggests that EMT may occur in pre-malignant tumors (i.e. nevus) before they enter the senescence state characteristic of these benign lesions. Based on this, EMT may be considered as a target not only of tumor progression but also of tumor initiation. The findings may have particular application in understanding the development of giant congenital nevi and their associated tumors.

28 Cilia Motility Studies Found to be an Effective and Efficient Screening Tool for Primary Ciliary Dyskinesia: Review of 111 cases from One Institute.

Anita Gupta M.D., Christopher M. Woods, Irene Hoffman, Department of Pathology, Cincinnati Children's Hospital, Cincinnati, OH

Background: Electron microscopy has been the universal gold standard method for diagnosing primary ciliary dyskinesia (PCD); however, applying this method on every cilia biopsy is time consuming and requires skilled evaluation. Despite normal motility studies and low clinical suspicion, electron microscopy is still asked to be performed. The objective of our study was to correlate the results of our cilia motility screening method with patient clinical history, degree of clinical suspicion, ultrastructural findings, and type of anesthesia (local or general) in order to improve the efficiency of primary ciliary dyskinesia work-ups.

Design: We evaluated 111 specimens collected between Jan 2009 and Sept 2010. The respiratory mucosal tissue for cilia motility studies was obtained and immediately submerged into room temperature RPMI media, prepared as wet preps and viewed with Normarski polarizing optics. Cases with cilium structural abnormalities (short and stubby) and/or abnormal cilia motility (asynchronous motility) were diagnosed as abnormal. Separate pieces of respiratory mucosal tissue was submitted in EM fixative then processed further. If the motility study was normal, the EM fixative tissue was embedded, and processed further at clinician's request only. At the time of the biopsy, the investigators were asked to fill out a worksheet for each patient pertaining to clinical history, associated anomalies, degree of suspicion of primary ciliary dyskinesia (low or high), and type of anesthesia (local or general).

Results: Over 90% of the specimens were obtained via curettage (rhinoprobe). Children with recurrent pneumonia (52%), rhinosinusitis (69%), chronic productive cough (72%), atypical asthma (46%), otitis media with effusion (45%), and continuous rhinorrhea since birth (21%) were most likely to be biopsied. Five percent of the children had heart abnormalities; 60% of these children were found to have ultrastructural cilia abnormalities consistent with primary ciliary dyskinesia. There was a high clinical suspicion of PCD in 36%

(40/111) of the cases. Additional 5 cases with low suspicion were processed based on either abnormal motility synchrony, or short, stubby nature of the cilia on motility study. Table 1: Compares Cilia Motility with Ultrastructural Findings Not diagnostic Normal Secondary Changes Suggestive of PCD Total Inadequate 3 (50%) 0 3 (50%) 0 6 Normal 4 (25%) 5 (31%) 7 (44%) 0 16 Abnormal 2 (8%) 4 (17%) 13 (58%) 4 (17%) 23 9 9 23 4 45

Conclusion: Although the current concept is to run EM on all adequate cilia biopsies, our institutional results suggests that cilia motility studies are an effective and efficient screening tool which when abnormal, can detect ultrastructural findings suggestive of PCD (17%). In addition, the method also reduces overall labor, screening, and turn around time.

29 The Utilization of Fine Needle Aspiration in Clinical Decision-Making in Pediatrics

DS Cleveland, S Cope-Yokoyama, Children's Medical Center Dallas and UT Southwestern Medical Center, Dallas, TX.

Background: Fine needle aspiration (FNA) is a useful diagnostic tool for clinicians and has been increasingly employed in the pediatric population. It is a noninvasive diagnostic procedure, provides an alternative to surgical biopsy, and may be used to determine if surgical management is indicated. The knowledge gained from an FNA diagnosis is valuable in making subsequent clinical decisions.

Design: A retrospective review of all FNA procedures performed at Children's Medical Center Dallas from August 1, 2008 to August 1, 2010 was undertaken. All patients who underwent an FNA and who had material sufficient for diagnosis were included in the study. The following data were gathered on each patient: age, sex, type of procedure (pathologist-, surgeon-, or radiologist-performed), anatomic location, correlation between FNA diagnosis and diagnosis of surgical follow-up, if applicable, and the clinical decision made based on the FNA diagnosis.

Results: Eighty-three patients underwent FNA procedures during the reviewed time period, and all had material sufficient for diagnosis except for six patients; seventy-seven patients were included in the study. There were 47 female and 30 male patients with an age range of 1 - 19 years (mean 8 years). There were 48 (62%) FNA procedures performed by a pathologist, 6 (8%) FNA procedures performed by a surgeon, and 23 (30%) FNA procedures performed by an interventional radiologist. Of the interventional radiology FNA procedures, 19 were ultrasound-guided and 4 were CT-guided. The anatomic locations of the procedures were as follows: 42 (55%) head and neck, 27 (35%) lymph node, 6 (8%) chest, and 2 (2%) other. Most clinicians opted to monitor patients (n=45, 58%) after FNA procedures. In 4 patients (5%), surgical excision was recommended but parents preferred to monitor; none of these patients have undergone surgery. In two patients (3%), clinicians chose subsequent surgical biopsy to further characterize an FNA diagnosis of sarcoma, and sixteen patients (22%) underwent surgical excision. No discrepancies were

noted between the FNA and surgical biopsy/excision diagnosis. Other courses of action included: chemotherapy in 5 patients (6%), medical therapy such as antibiotics or hormone therapy in 4 patients (5%), and repeat FNA in 1 patient (1%). There was no significant difference in clinical decision making between pathologist- and interventional radiologist-performed FNA procedures, or between the different referring services (ENT, Surgery, Pediatrics, other).

Conclusion: Fine needle aspiration is a helpful tool for pediatric clinicians to use in the decision-making process and can potentially decrease the overall number of surgical procedures. In our experience FNA procedures have allowed clinicians to simply monitor patients, to use subsequent surgical intervention judiciously, and in some cases, to institute therapy without surgical intervention. In patients undergoing subsequent surgical intervention, the FNA diagnosis correlates with the diagnosis made by subsequent intervention.

Poster Presentations

30 Detection Of Bartonella henselae In Formalin-Fixed, Paraffin-Embedded Tissues, By A Hemi-Nested Real-Time Polymerase Chain Reaction, Targeting Citrate Synthetase (glTA) Gene; A Children's Hospital Experience.

JN Punia, AD Rector, CR Webb, PA Revell, Division of Molecular Microbiology, Department of Pathology, Texas Children's Hospital, Houston, Texas.

Background: Cat-scratch disease (CSD), a necrotizing granulomatous lymphadenitis, is caused by Bartonella henselae, a gram-negative pleomorphic bacillus transmitted to humans by the scratch of a cat. Although CSD is a self-limited infection in immunocompetent patients, in immunocompromised patients it can result in systemic, sometimes life-threatening disease with multiple internal organ involvement. Traditional diagnostic practices for detection of Bartonella infection include serological antibody assay and histological evaluation, including a Warthin Starry/Steiner stain which may give false negative results. The aim of our study was to detect the presence of Bartonella spp in formalin-fixed, paraffin-embedded (FFPE) tissue as an ancillary test for diagnosis of CSD.

Design: After an initial validation, archived cases with necrotizing granulomatous inflammation, suspicious/suggestive of CSD were identified and cases with similar features were included. Two to five additional 10 µM unstained sections were obtained from a representative FFPE block of each case. Sections were deparaffinized, DNA was manually extracted using Qiagen Qiaamp DNA mini kit, and amplified by end-point PCR targeting the citrate synthetase (glTA) gene. The amplicon obtained was used as template for the second hemi-nested real-time PCR. Melting curve analysis with specific FRET probes was used to confirm Bartonella spp.

Results: The total cases with features suspicious of CSD were 48, with ages ranging from 5 months to 17 years. Of these, 19 tested positive for Bartonella spp by PCR, mostly involving the lymph nodes, 3 involving lung

tissue and 1 case of parotid gland. Of the positive cases, 6 cases were historical archived cases, with no clinical notes retrievable through the electronic data system. Serologic results from the remainder of the 13 positive cases were as follows: 1 was positive by serology, 2 showed elevated Bartonella IgG levels, suggesting a past exposure/infection, 1 was negative, 4 cases had no serological studies done and 5 were consult cases with no information regarding the serological studies. Warthin starry/Steiner stain was performed and negative in 6 cases. For the remaining positive cases, either the stain was not performed, or the information was not available. One case had positive serology, but was negative by PCR. **Conclusion:** Targeting the citrate synthetase (glfA) gene for the molecular identification of Bartonella spp provides a rapid diagnostic test as a supplemental tool for the histological diagnosis of CSD. We were able to detect 9 cases with negative or unknown serology/special stain. The detection of Bartonella spp in FPPE tissues by molecular techniques is helpful for diagnosis and hence subsequent appropriate treatment of patients to avoid increased morbidity.

31 Placental Weight Below the 10th Percentile is Associated with Intrauterine Fetal Demise at 35 weeks or Greater Estimated Gestational Age: A Retrospective Autopsy Review.

Matthew P. Thompson, DO, MS Sarah Johnson-Welch, MD, Children's Medical Center Dallas, TX

Background: Approximately 25,000 cases of intrauterine fetal demise (IUFD) are reported every year, representing 60% of all perinatal mortality in the US. Advancements in ultrasound technology have made it possible to accurately assess placental size and volume in utero and with good correlation with actual placental weight after delivery. Sonographically-assessed decreased placental volumes in the second trimester have been associated with the subsequent delivery of small for gestational age infants, extreme prematurity, gestational hypertensive disorders, stillbirth, and chronic villous inflammation. However, the association of third trimester placental volume and weight with increased risk of intrauterine death has not been studied.

Design: Autopsy reports of all intrauterine deaths at our institution from 2003-2007 (n = 255) were reviewed for all diagnoses and findings including placental weight, fetal weight, placental or umbilical cord abnormalities, evidence of fetal hypoxia, infection, and gestational age at time of intrauterine death. To gauge the association of placental weight with near-term intrauterine deaths, the cases were grouped into greater than or equal to 35 weeks estimated gestational age (EGA) and less than 35 weeks EGA, within a range of 18-43 weeks EGA.

Results: For the entire series, placental weight below the 10th percentile was associated with fetal weight below the 10th percentile ($P = 0.0006$) and placental infarction ($P < 0.0001$). When the cases were grouped by EGA, 78 of 114 intrauterine deaths at or beyond 35 weeks EGA had placental weights below the 10th percentile (68.4%), while 35 of 141 intrauterine deaths less than 35 weeks EGA had placental weights below the 10th percentile

(24.8%, $P < 0.0001$). Low fetal weight, placental infarction, placental chronic inflammation, evidence of fetal hypoxia, umbilical cord abnormalities, placental abruption, and acute fetal or placental infection were not associated with IUFD at 35 weeks EGA or greater.

Conclusion: In our retrospective autopsy data, placental weight below the 10th percentile for EGA is independently associated with IUFD occurring at 35 weeks EGA or greater. The absence of other risk factors associated with IUFD at 35 weeks EGA or greater implies that placental weight below the 10th percentile may be a significant contributor to late-gestation fetal deaths. Our data suggests that ultrasonographic placental assessment later in gestation has the potential to benefit obstetricians in identifying a unique population at increased risk of late term IUFD.

32 Analysis of Epithelioid Angiomyolipoma using SNP Copy Number Arrays Showing LOH of 16p

SC Shulman, W Tang, F David, HM Katzenstein, S Langess, CR Abramowsky, M Bouzyk, MR Rossi, BM Shehata, Children's Healthcare of Atlanta, Atlanta, GA; Emory University School of Medicine, Atlanta, GA.

Background: Renal angiomyolipoma (AML) is characterized as a mesenchymal neoplasm that is composed of dysmorphic blood vessels, smooth muscle, and adipose tissue. Epithelioid AML, a rare subset of AML, is characterized by cells varying in size with abundant acidophilic cytoplasm. The cells may be mono- or multinucleated and contain round to oval nucleoli with macronucleoli. Epithelioid AML is considered as a malignant neoplasm, often behaving aggressively with a propensity for metastasis. All subsets of AML are commonly associated with multiple hereditary diseases including tuberous sclerosis, von Recklinghausen disease, von Hippel-Lindau syndrome, and autosomal dominant polycystic kidney disease. Detection of molecular events in AML tumors, particularly those arising in patients at risk of one of these diseases, may have significant impact on diagnosis, management and treatment of these individuals.

Design: We reviewed over 300 cases of renal tumors from 1975-present from Children's Healthcare of Atlanta. We found five cases of AML and reviewed all subsequent histologic, demographic and clinical data. Two epithelioid AML specimens were analyzed using Illumina Humana Omni1_Quad SNP Arrays to determine copy number and LOH events.

Results: To date, this is the first study using SNP copy number arrays to analyze epithelioid AML. In both of the tumors analyzed, LOH of 16p was detected as the sole abnormality. Although, the LOH events were not identical (~3.5 Mb and ~28.6 Mb) in the two tumors, both LOH events contained the TSC2 gene (16p13.3).

Conclusion: Using SNP copy number arrays, LOH of 16p was the sole abnormality detected in the two epithelioid AML tumors tested. Subsequent follow-up of these cases revealed that both patients had been diagnosed with tuberous sclerosis, an autosomal dominant disorder caused by mutations in either the TSC1 (9q34) or TSC2 genes. These data suggest that analysis of LOH and copy number changes in AML and

other renal tumors using SNP copy number arrays may be useful in identifying individuals at risk for genetic disorders. Further studies will be necessary to determine if SNP copy number arrays can identify other changes in epithelioid AML tumors in addition to LOH of 16p.

33 Placental Histologic Criteria for Diagnosis of Cord Accident: Sensitivity and Specificity

WD Ryan, N Trivedi, Y Lacoursiere, MM Parast, University of California, San Diego School of Medicine, San Diego, CA.

Background: Stillbirth, defined as intrauterine fetal demise (IUFD) after 20 weeks gestational age, is highly understudied. Many stillbirths have no apparent cause even after a full autopsy and placental examination. "Cord accident" (or compromised umbilical blood flow) as a cause of stillbirth is under-reported, mainly due to the lack of diagnostic criteria. We have previously established histologic criteria for the diagnosis of cord accident, based on fetal vascular pathology in the placenta. In the current study, we set out to test the sensitivity and specificity of these criteria by reviewing an independent set of stillbirth cases.

Design: We reviewed all singleton stillbirths where a full autopsy and placental examination were performed at our hospital over the past 10 years. All cases for which placental slides were available (at least one section each of umbilical cord and membrane roll and two sections of placental disc) were retained in the study and reviewed. In total, we reviewed placental slides of 26 cases (where "cord accident" was deemed the cause of death) and 62 controls (where the cause of death was anything other than cord accident). The following histologic changes were noted: (1) dilated fetal vessels; (2) thrombosis in fetal vessels; and (3) avascular or near-vascular chorionic villi. The former two changes were noted in the umbilical cord, chorionic plate, and/or stem villous vessels. We defined minimal criteria as the presence of dilated and thrombosed fetal vessels, while the additional presence of avascular villi satisfied the complete criteria.

Results: Of the 62 stillbirth controls with a cause of death other than cord accident, 14 (23%) met the minimal criteria (specificity 77%) and only 6 (10%) met the complete criteria for cord accident (specificity 90%). In contrast, of the 26 cases with a cause of death related to cord accident, 16 met the minimal criteria (sensitivity 62%) and 12 met the complete criteria (sensitivity 46%).

Conclusion: Minimal criteria identify the majority of stillbirths caused by cord accident. The additional finding of avascular chorionic villi increases the specificity of these criteria, but does not identify more cases. This study confirms the utility of these criteria for the diagnosis of cord accident and further stresses placental examination in the evaluation of stillbirths.

34 Anaplastic Wilms' Tumor: Molecular Analysis of 7 Cases Using SNP Copy Number Arrays

SM Langness, HM Katzenstein, W Tang, F David, M Bouzyk, MR Rossi, CA Abramowsky, SC Shulman, BM

Shehata, Children's Healthcare of Atlanta, Atlanta GA; Emory University School of Medicine, Atlanta GA.

Background: Wilms' tumor is characterized by an abnormal proliferation of primitive embryologic cells of the kidney. It is classified as favorable or unfavorable histology depending on the presence or absence of focal or diffuse anaplasia. This is an important distinction as anaplastic Wilms' (unfavorable) is associated with a worse prognosis and requires more aggressive treatment. There is a paucity of information regarding the genetic/molecular basis of Wilms' tumor in general and anaplastic Wilms' in particular.

Design: We reviewed over 300 cases of renal tumors from 1975-present from Children's Healthcare of Atlanta. Nineteen cases of anaplastic Wilms' tumor were identified and corresponding histology, demographics and clinical data was reviewed. Frozen tissue was available from 7 of the cases and Illumina Humana Omni1_Quad SNP Arrays analysis was done to determine copy number and LOH events.

Results: Of the 7 cases that underwent SNP array analysis, 2 were stage V, 1 was stage IV, 2 were stage III and 2 were stage I. Except for the 2 patients with stage I, all remaining cases demonstrated a heterogenous loss of heterozygosity (LOH) in several chromosomes. Interestingly, 2 cases demonstrated LOH of 17p (p53) who presented as stage IV and V. The stage V patient died 14 months after initial diagnosis with extensive metastatic disease. The stage IV patient has remained in remission 13 months after completing chemotherapy.

Conclusion: Loss of heterozygosity in the p53 region has been associated with several childhood tumors. It appears that it is also associated with unfavorable outcomes in patients with anaplastic Wilms' tumor. Further studies and longer follow-up will be important to verify the significance of this mutation in Wilms' tumor patients.

35 Restrictive Cardiomyopathy: A Review of Eight Cases, Including Two Novel Congenital Cases

JH Sacks, SC Shulman, WT Mahle, CR Abramowsky, BM Shehata, Children's Healthcare of Atlanta, Atlanta, GA; Emory University School of Medicine, Atlanta, GA.

Background: Restrictive cardiomyopathy (RC) is a rare entity in the pediatric population. While endemic in various regions, it is quite uncommon in the developed world. It is characterized most notably by endocardial fibroelastosis and/or prominent interstitial fibrosis. These pathologic changes result in stiffness of the myocardium with loss of elasticity and eventual heart failure. This can be a devastating condition with poor outcomes without heart transplantation or significant intervention. Although genetic predisposition has been identified, there have been no previous reports of congenital appearance of this disease.

Design: We reviewed over 400 cases of cardiomyopathy from 1992-2010 from Children's Healthcare of Atlanta. All histologic, demographic and clinical data were reviewed.

Results: Among the 400 cases of cardiomyopathy, we identified eight cases of RC which were both clinically

and pathologically differentiated from Left Ventricular Noncompaction Cardiomyopathy (LVNC). One patient was identified at birth and another at less than three months of age, qualifying both as congenital cases. No additional congenital cardiovascular anomalies were detected in the eight patients. All patients underwent cardiac biopsy and/or transplant. The patient diagnosed at birth had the most expansive endocardial fibroelastosis and the patient diagnosed at 75 days displayed the most extensive interstitial fibrosis.

Conclusion: Restrictive Cardiomyopathy is extremely rare in the pediatric population and according to our knowledge; we report the first two congenital cases of RC. Our observations strongly suggest a genetic component to RC. Although LVNC can often be misdiagnosed as RC, the use of strict clinical and pathological criteria can help minimize diagnostic error to ensure that RC can be correctly classified and studied. Further molecular analysis is warranted to characterize this rare subset of cardiomyopathy.

36 The Spectrum of Acute and Healed Phase Cardiovascular Lesions in Kawasaki Disease.

M Warren, KS Thompson, M Melish and DL Kearney, Kapiolani Medical Center for Women & Children, Honolulu HI; Texas Childrens Hospital, Houston TX

Background: Kawasaki' disease (KD) is an acute vasculitis syndrome that affects young children of all races with a world wide distribution. The annual incidence is 216.9 cases and 9-45.9 cases per 100,000 children under 5 years of age in Japan (highest in the world), and the United States (US), respectively. The etiology remains elusive, although an infectious agent has long been suspected. Recent studies suggest an abnormal immunologic response in a genetically susceptible host. KD causes a systemic panarteritis predominantly affecting small to medium sized vessels. Involvement of the heart and coronary arteries may lead to the dreaded complication of coronary aneurysms (CA), thrombosis, myocardial infarction (MI) and death. Since the introduction of intravenous immunoglobulin (IVIG) to the treatment regimen, specifically within 10 days of fever onset, the prevalence of cardiac lesions has decreased. The incidence of CA has decreased from 25% to 3-8% and overall mortality has decreased from 2% to less than 0.1%. Despite the potential for successful early IVIG intervention, reports suggest that nearly 30% of KD patients may have delayed diagnosis and not all patients respond to therapy. In both Japan and the US, KD remains the most common cause of acquired heart disease in childhood.

Design: We present the autopsy findings of 6 children, aged 2- 23 mos, including 3 Japanese, 2 Caucasian and 1 of unknown race/nationality. No child received IVIG therapy. Three died in the acute symptomatic phase (9 - 18 dys) and 3 died after resolution of acute symptoms (healed phase, 23 dys - 10 wks). Complete autopsies were performed in all cases. The autopsy reports, all available tissues and/or slides were reviewed. This included glass slides from all organs in 4/6 cases and the gross hearts and heart slides only in 2/3 cases with CA.

Results: All acute phase deaths had coronary and systemic arteritis with coronary thrombi in 1/3. Myocarditis, present in 2/3 cases, was extensive in one case with severe valvulitis. The healed phase deaths all had giant CA involving multiple vessels with acute thromboarteritis in 2/3 cases and acute MI in 1/3. The third patient had extensive remote, organizing, partially recanalized occlusive thrombi with extensive, biventricular remote MI. In both acute and healed cases, eosinophils were focally increased in areas of myocarditis and arteritis. In all cases, the cardiac lesions directly contributed to patient demise. One acute phase death also had sepsis.

Conclusion: This study demonstrates a continuous spectrum of active to healed cardiovascular postmortem findings in KD, in the absence of IVIG. The acute phase of KD is associated with myocarditis and widespread coronary and systemic arteritis. One case with coronary thromboarteritis and no myocarditis had the longest acute phase duration, suggesting a transition phase. Healed phase KD all had giant CA with mild arteritis in 2/3 and remote MI with extensive fibrosis in the oldest duration case. These findings remain relevant to KD untreated or nonresponsive to IVIG.

37 Unusual Pathogens and the Pediatric Autopsy.

D Drehner, M Turner, P Bakken, Children's Hospitals & Clinics of Minnesota.

Background: The identification of unexpected pathogens in a well population requires an index of suspicion and persistence. Sampling of multiple sites with conventional viral and bacterial cultures is necessary to exclude those pathogens. The high sensitivity and specificity of molecular techniques give the possibility of establishing diagnoses when cultures are negative. Light microscopic evaluation of tissue and cytology samples is key to identifying the pathogen and/or the pattern of tissue injury associated with the pathogen.

Design: This is a case series of autopsy cases from the period 1/1/2005 to 10/18/2010. During that time 506 autopsies were performed at Children's Hospitals and Clinics of Minnesota. Criteria for selection were: the patient was previously healthy; the infectious agent was unsuspected and/or unlikely to occur in individuals with the patient's demographic profile and the hospital course was less than one week.

Results: Three cases were found. A seven year old female with a clinical diagnosis of bacterial meningitis was found to have meningo-encephalitis due to *Naegleria fowleri*. A twenty one month old female admitted with fever, thrombocytopenia and a whole body petechial rash was diagnosed by PCR with *Rickettsia rickettsii* infection. Those infectious agents had rarely or not previously been reported to cause disease in Minnesota. The third was a case of massive myonecrosis secondary to *Clostridium septicum* in a 13 year old female without a history of trauma or underlying disease.

Conclusion: Extensive sampling and use of multiple testing modalities lead to identification of the pathogens, excluded other more common agents, provided important information to the community and gave the families

explanations for the deaths. A key to accomplishing those ends was histology staff trained to effectively assist in the prosecution and the rapid processing/distribution of samples. Support from the Minnesota Department of Health facilitated rapid testing for unusual pathogens.

38 Correlation of Immunohistochemical CD133 Expression with Histological Subtype in Pediatric Synovial Sarcomas

ML Calicchio, J Terry, Department of Pathology, Children's Hospital Boston and Harvard Medical School, Boston, MA.

Background: Synovial sarcoma is a soft tissue malignancy of adolescents and young adults characterized by a histological spectrum ranging from mesenchymal to epithelial. A subset of synovial sarcoma cells expresses the neuroepithelial and neural cancer stem-like cell marker CD133, implying a role for primitive CD133+ cancer stem-like cells in synovial sarcoma pathogenesis (AJMM; 18(2): 159). The less differentiated mesenchymal component of synovial sarcoma would seem the likely source of the CD133+ subpopulation; however, luminal membrane CD133 expression is also reported in well differentiated glandular tissues and CD133 expression in synovial sarcoma may be instead related to epithelial differentiation. Comparing CD133 expression in mesenchymal and epithelial components of synovial sarcomas would help identify the source but has thus far been hindered by a lack of an effective protocol for immunostaining formalin fixed paraffin embedded (FFPE) tissue. Here, immunohistochemical detection of CD133 in FFPE synovial sarcoma is optimized and the expression patterns of CD133 in pediatric monophasic and biphasic specimens are examined.

Design: Archival FFPE tissue from three monophasic and two biphasic synovial sarcomas are retrieved from the archives of Children's Hospital Boston. All tumors are t(X;18) positive by cytogenetic analysis. Immunohistochemical detection of CD133 is performed using a 1:25 dilution of anti-CD133 (clone C24B9) on a Ventana Discovery XT automated immunostainer with modified CC1 epitope retrieval protocol. Positive staining is defined as membranous pattern with or without a cytoplasmic component. Liver bile duct epithelium and immature teratoma neuroepithelium are used as positive staining controls and smooth muscle as a negative staining control.

Results: The biphasic tumors show individual CD133+ positive cells in both the spindle and epithelioid components with occasional clusters and rare glandular structures with luminal membranous staining similar to the positive controls. The epithelioid cells show more intense staining and are more abundant than their spindled counterparts. Individual CD133+ cells are also present in two of the three monophasic tumors but are much less frequent compared to the biphasic tumors. The positive staining controls show luminal membranous staining and the negative control shows no staining, as expected.

Conclusion: CD133 expression in synovial sarcoma appears to be positively correlated with epithelial differentiation.

39 The Utility of Calretinin Immunohistochemistry in the Primary Diagnosis of Hirschsprung Disease - A Retrospective Review of 78 Cases with Emphasis on Diagnostic Pitfalls in the Interpretation of Calretinin Immunoreactivity

KTE Chang, WS Hwang, KK Women's and Children's Hospital, Singapore

Background: Calretinin immunohistochemistry has been described as an adjunctive diagnostic technique in the primary diagnosis of Hirschsprung Disease (HSCR). Suction rectal biopsies from non-HSCR patients show calretinin immunoreactivity of small mucosal and submucosal nerves. Those from HSCR-patients show absent immunolabeling of these nerves. Calretinin immunohistochemistry may therefore serve as an alternative to acetylcholinesterase histochemistry in the primary diagnosis of HSCR by suction rectal biopsies. This study is a retrospective review of the utility of calretinin immunohistochemistry in the primary diagnosis of HSCR.

Design: Rectal suction biopsies obtained for the primary diagnosis of HSCR in our institution incorporated routine calretinin immunohistochemistry from 2007 onwards. These biopsies were reviewed in relation to pull-through resection specimens when HSCR was diagnosed, which served as the gold standard for the diagnosis of HSCR. Diagnosis of HSCR was made if at least one adequately sized biopsy >3mm in diameter containing submucosa of at least equal thickness as mucosa did not contain ganglion cells in >100 levels examined. Concurrent calretinin staining was performed for every biopsy.

Results: 78 patients over a period of four years had rectal suction biopsies to assess for HSCR. 50 patients had biopsies which contained ganglion cells, excluding HSCR. Calretinin stains in these biopsies showed confluent dark granular reactivity of small-calibre nerves in the superficial submucosa and lamina propria. 3 patients had morphologically low biopsies; repeat biopsies were not obtained. 25 patients had biopsies which met diagnostic criteria for HSCR; of these patients, 13 had pull-through resections, and the diagnosis of HSCR was confirmed in all 13 patients. The calretinin stains of the 25 patients with HSCR had no immunoreactivity in small submucosal and mucosal nerves and nerve fibres. Cytoplasmic calretinin reactivity of mast cells was a consistent feature. One patient required a repeat biopsy before the diagnosis of HSCR was made - the reason for the initial false-negative result was the presence of punctate calretinin immunoreactivity in large submucosal nerves. A potential false-positive result was seen in a set of non-HSCR biopsies was processed for frozen section diagnosis; the calretinin immunoreactivity was markedly diminished in these previously frozen biopsies.

Conclusion: Calretinin immunostaining is a reliable adjunct in HSCR diagnosis. Potential pitfalls need to be recognised to prevent false-negative/positive results.

These include: (i) punctate immunoreactivity of large submucosal nerves resulting in a false-negative result in a patient with HSCR, (ii) markedly diminished calretinin immunoreactivity in previously frozen biopsies, and (iii) mast cell immunoreactivity which should not be misinterpreted as nerve immunoreactivity, but which is a useful internal positive control.

40 Significance of Staphylococcus Coagulase Negative Culture Results in Perinatal Postmortem Examinations

Mai He, C. James Sung, Jinyi Weng, Halit Pinar, Department of Pathology, Women & Infants Hospital of RI and Brown Medical School

Background: Coagulase-negative staphylococci (CoNS) are one of the most common pathogens isolated from blood culture in neonatal intensive care units (NICU). Pathologists are often faced with the dilemma of distinguishing true CoNS infection from skin contamination in part due to the low virulence factors of the organism. Until now we could not find any pathological study of CoNS infection in postmortem (autopsy) materials. The purpose of this study is to study the histopathological-microbiological correlations of CoNS, including Staphylococci epidermidis in postmortem examinations.

Design: Achieves of perinatal (stillborn and neonatal) postmortem examination between the year of 2000 to September 2010 were searched for positive (heart) blood or (lung) tissue culture for CoNS or Staphylococcus epidermidis. Histopathological evidence of infection/inflammation in placentas, feti or neonates were correlated with postmortem culture results.

Results: During the study period 1197 cases of postmortem examinations were performed in our hospital. Seventy two (72/1197, 6.0%) cases yielded 84 positive cultures for CoNS or Staphylococcus epidermidis, including 38 (52.8%) stillborn and 34 (47.2%) liveborn. Culture results when stratified to stillborn/liverborn with association to histological evidence of infection were summarized in table 1. In stillborn cases, the gestational age ranged from 14 weeks to term, with 35 (92.1%) preterm (< 37 weeks) cases. There were 20 (20/38, 52.6%) cases demonstrating histological evidence of fetal infection and/or placental inflammation with 13 (13/38, 34.2%) cases showing pure cultures. In liveborn group, there were 31 (31/34, 91.2%) preterm infants. The neonates lived less than 1 hour to 158 days. Twenty-four cases (24/34, 70.6%) demonstrated histological evidence of neonatal infection such as pneumonia, including 12 (12/34, 35.3%) cases with pure cultures.

Tbl 1. Postmortem Exam Cases w/ Culture Positive for CoNS
Histological evidence

of infection	Stillborn			Liveborn		
	< 28 w	29-36 w	Term	<= 48 h	48h - 7d	> 7d
Present	15	2	3	6	4	14
Absent	12	6	0	3	2	5
Total	38			34		

Conclusion: During the ten-year period, 34.2% in stillborn and 35.3% of the liveborns had positive

postmortem cultures for CoNS associated with morphological evidence of infection. Our results not only confirmed that CoNS in the NICU setting is still a significant pathogen, but also demonstrated that in the stillborn it plays a significant role as an infectious cause of death.

41 Sensitivity and Specificity of Finding of Multinucleate Trophoblastic Giant Cells in Decidua in Placentas from High-risk Pregnancies

Jerzy Stanek, Division of Pathology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH

Background: Increased decidual multinucleate trophoblastic giant cells (MTGC) were described as a feature of maternal vascular underperfusion. This analysis intends to retrospectively check the MTGC sensitivity and specificity for clinical conditions and placental lesions associated with fetal and placental hypoxia in placentas from high-risk pregnancies.

Design: This is a retrospective analysis of the author's placental database of ≥ 20 week pregnancies. Selected clinical and placental parameters of 375 consecutive cases with clusters of MTGC (at least 3 cells with at least 3 nuclei) in the decidua study group SG] were compared to all remaining 2674 placentas concurrently studied control group, CG]. Statistical evaluation included the analysis of variance, correlation coefficient, or Yates χ^2 .

Results: MTGC were found in 12.3% of placentas. Gestational age was 33 ± 6 vs 34 ± 5 , and placental weight 353 ± 181 vs 400 ± 181 in the SG and the CG (average \pm SD), respectively. The MTGC prevalence negatively correlated with gestational age ($R = -0.56$), peaking at the turn of the second and the third trimester of pregnancy, ant plateauing out afterwards. The sensitivity and specificity of MTGC (%) for selected clinical and placental factors were as follows, respectively (bold font: $p < 0.05$ for differences between the SG and the CG; prevalence in the CG not shown): pregnancy-induced hypertension 2 vs 18, preeclampsia 25 vs 34, chronic hypertension 2 vs 12, diabetes mellitus 5 vs 11, maternal substance abuse 7 vs 16, abnormal Dopplers 6 vs 26, perinatal mortality 17 vs 15, fetal growth restriction 19 vs 20, induction of labor 13 vs 21, cesarean sections 45 vs 13, erythroblasts in fetal blood 11 vs 17, deep membrane meconium penetration 11 vs 16, villous infarction 15 vs 21, retroplacental hematoma 8 vs 17, intravillous hemorrhage 4 vs 23, hypertrophic decidual arteriopathy 30 vs 21, atherosclerosis of spiral arterioles 9 vs 25, membrane lamellar necrosis 32 vs 18, diffuse patterns of hypoxic placental injury 25 vs 24 (preuterine 4 vs 14, uterine 13 vs 29, postuterine 7 vs 25), membrane and disc microscopic cysts 13 and 13 vs 17 and 24, and excessive amount of extravillous trophoblasts 19 vs 41.

Conclusion: The MTGC prevalence and its gestational age profile parallels those of the excessive amount of extravillous trophoblasts, most likely reflecting an increased fusion of excessively numerous extravillous trophoblasts secondary to hypoxia. The specificity of MTGC for most clinical conditions and placental lesions is higher than its sensitivity. Finding of MTG is not exclusively seen in uteroplacental malperfusion of

preeclampsia, as it is also associated also with the postuterine pattern of hypoxic placental injury and they are also seen in other types of high-risk pregnancy like diabetes mellitus or chronic hypertension. Furthermore, 2/3 of cases of preeclamptic placentas did not show the MTGC.

42 Interfollicular Hodgkin Lymphoma in Children: an Uncommon Mimicker of Reactive Lymphadenopathy

Mariana M. Cajaiba, MD; Samir Kahwash, MD, Department of Pathology, Nationwide Children's Hospital, Columbus, OH

Background: Interfollicular Hodgkin lymphoma (IFHL) has been described as a histopathological variant of Hodgkin lymphoma (HL) characterized by subtle morphological features that may mimic follicular hyperplasia under low-power histopathological examination. Limited published data points towards a similar clinical course to well-characterized subtypes of classical HL. Although IFHL is mentioned under the 2008 WHO classification as a morphological pattern often seen in the mixed cellularity subtype, its prevalence and clinicopathological spectrum have not been fully characterized.

Design: A retrospective search for surgical pathology specimens diagnosed as HL from 1990 through 2010 was performed. Final diagnosis and microscopic description from all diagnostic reports were reviewed; cases with a diagnosis of IFHL or morphological features suggestive of the latter were selected. Archival H&E and immunohistochemical slides and medical charts were retrieved for review.

Results: 112 cases of HL were diagnosed between 1990 and 2010. Among those, 4 (3.6%) IFHL cases were identified; none had had a previous biopsy. Complete clinical information was available for 3/4 cases (summarized on Table 1). The most common anatomical site was cervical (3/4), followed by supra-clavicular (2/4) and mediastinal lymph nodes (2/4). Low-power microscopic examination in all cases showed a preserved follicular architecture in most areas, highlighted by CD3/CD20 stains, with mild expansion of the interfollicular zones. Short fibrous bands without a nodular pattern were focally present in two cases. Two cases showed mild sinus histiocytosis. On high magnification, scattered CD15/CD30-positive, LCA-negative variants of Reed-Sternberg cells were found in the interfollicular areas of all cases, often associated with clusters of eosinophils, histiocytes and plasma cells.

Table 1

Case	Age	Sex	Stage	B-symptoms	Complete Remission	Follow-up (years)
1	14	F	II	YES	YES	11
2	17	M	I	NO	YES	5
3	14	F	II	YES	YES	4
4	4	M	II	?	?	?

Conclusion: 1) IFHL appears to be an uncommon morphological variant of pediatric HL based on a retrospective search from our surgical pathology files. 2) Our limited sample number precludes conclusions on demographic and clinical characteristics. However, a

favorable course is suggested by the early stages and excellent clinical outcomes observed. 3) The subtle histopathological findings at low-power examination may mimic a reactive lymphadenopathy, especially in pediatric pathology practice where a follicular pattern is almost always benign due to the rarity of follicular lymphoma. 4) Awareness of this entity during routine examination of lymph nodes is essential to avoid diagnostic pitfalls.

43 Comparison of S-Phase in Precursor B-Lymphoblastic Leukemia of Childhood between Initial Diagnosis and Relapse

J Shao¹, F Castro-Silva², A McGranahan¹, X Liang^{1,2}, ¹The Children's Hospital, CO, ²University of Colorado Denver School of Medicine

Background: S-phase fraction (SPF) is an indicator of cell proliferation. High grade neoplasms often have a higher SPF than low grade ones. Precursor B-lymphoblastic leukemia (PBL) is the most common malignant neoplasm in childhood. PBLs at relapse are more difficult to treat than those at initial diagnosis. Whether the cell proliferation rate has any significant change as disease relapses and the possible role of its change in tumor resistance to therapy at relapse is unclear in PBLs. In this study, we examined SPF in a series of pediatric PBLs by comparing the SPFs at the time of diagnosis with those at relapse.

Design: 26 cases of pediatric LL at The Children's Hospital, Colorado from 1998 to 2010 which had SPF data available at both diagnosis and relapse were evaluated. SPF was analyzed by flow cytometry. We compared the SPF between the time of diagnosis and the first relapse as well as the SPF between the first relapse and the second relapse.

Results: In most cases, SPF at relapse was elevated than SPF at the time of diagnosis, and it was further elevated at the second relapse as compared with it at the first relapse.

Table 1

	# of cases with elevated SPF	Mean of SPF elevation
Diagnosis - 1st relapse	22/26 (85%)	226%
1st relapse - 2nd relapse	8/10 (80%)	159%

Conclusion: In pediatric patients, SPF is frequently elevated as PBL relapses and appears to be further elevated as PBL relapses repeatedly. These findings suggest that the progressive increase of tumor cell proliferation may partially be responsible for a more aggressive clinical behavior in PBL at relapse.

44 The Utility of NeuN and PGP 9.5 Immunohistochemistry in the Diagnostic Workup of Neuroblastoma and other Small Round Blue Cell Tumors

DL Stockman, EV Zambrano, JA Jarzembowski, Medical College of Wisconsin, Milwaukee, WI

Background: Pediatric small round blue cell tumors (SRBCT) remain diagnostically problematic, despite advances in our knowledge of the origin of these lesions. While some SRBCT have diagnostically useful

immunostaining patterns (lymphomas and rhabdomyosarcomas) or pathognomonic chromosomal translocations (peripheral neuroectodermal tumors PNET] and desmoplastic small round cell tumors DSRCT]), others are not amenable to such highly specific studies. NeuN is a recently described neuronal-specific nuclear protein which is expressed in most post-mitotic neurons and some central nervous system tumors, such as gangliogliomas and central ganglioneuromas; medulloblastomas are typically negative, and most colonic ganglion cells are positive. We investigated whether NeuN would be superior, or a useful adjunct, to PGP 9.5 in discriminating neuroblastic tumors from other SRBCT.

Design: We analyzed a set of 32 well-characterized SRBCT obtained from our institution for which adequate amounts of formalin-fixed paraffin-embedded tissue were available, including 7 neuroblastomas, 5 embryonal rhabdomyosarcomas (ERMS), 4 alveolar rhabdomyosarcomas (ARMS), 4 PNET, 4 Wilms tumors, 3 synovial sarcomas, 4 acute lymphoblastic lymphomas (ALL), and 1 DSRCT. The patients ranged from 5 months to 18 years of age (mean 7 y) and included 17 males and 15 females. Immunohistochemistry was performed with antibodies specific for NeuN and PGP.5 using manual (NeuN) or automated (PGP 9.5) methods according to the manufacturers' standard protocols. Tumor staining was assessed for percentage of positive cells, strong versus weak intensity, and nuclear versus cytoplasmic distribution.

Results: Positive nuclear staining for NeuN was seen in 87% of neuroblastomas; staining was stronger and more frequent in the differentiating, gangliocytoid cells. All 4 Wilms tumors showed scattered small clusters of blastemal cells with intense NeuN staining. Also, 100% of synovial sarcomas, 75% of ARMS, and 60% of ERMS stained positively for NeuN, as did the only DSRCT; signals were nuclear except for the ERMS, which also had cytoplasmic positivity. All cases of ALL and all but one of the PNETs were negative for NeuN. In contrast, only the neuroblastomas, synovial sarcomas, and Wilms tumors were positive for PGP 9.5; most had both nuclear and cytoplasmic signals.

Conclusion: NeuN is a less specific immunohistochemical marker than PGP 9.5; NeuN was positive in all PGP 9.5-positive tumors, as well as in the PGP 9.5-negative rhabdomyosarcomas. Contrary to initial assumptions that it would be highly specific for post-mitotic neurons and mature neuronal neoplasms, in neuroblastomas, NeuN stained both poorly differentiated neuroblasts and differentiating gangliocytoid cells, albeit to different degrees. Immunohistochemical positivity for either NeuN or PGP 9.5 serves well to exclude ALL from the differential diagnosis of SRBCT.

Background: Extracorporeal membrane oxygenation (ECMO) is a life saving therapy for infants and children with cardiac and respiratory failure, but is frequently complicated by hemostatic derangement. Symmetrical peripheral gangrene (simultaneous ischemia in multiple limbs without evidence of large artery occlusion) is a rare condition usually associated with disseminated intravascular coagulation (DIC) and sepsis. However, it has not been previously described in patients on ECMO.

Design: Four patients at our children's hospital developed symmetrical peripheral gangrene on ECMO following cardiac surgery, and subsequently expired and came to autopsy, over a two-year period (2008-2010). Clinical history, ECMO history, physical examination findings and laboratory studies were examined. Gross and microscopic autopsy material was reviewed.

Results: Patients ranged in age from 11 days to 13 years. ECMO duration was 11-22 days, and four-limb ischemia was present for 2-4 days prior to death. In two patients, onset was rapid, with development of nonblanching ecchymoses or cold, pulseless limbs over less than a day. In the other two, ischemic changes began as focal lesions and gradually spread. Two patients were septic (one with Serratia and Candida, the other with Aspergillus), while the others had negative antemortem and postmortem cultures. Three had evidence of other end-organ damage (kidney, brain, and/or liver). All four were anticoagulated with heparin, but two were changed to argatroban when ischemia developed. HIT antibody testing was negative. Two patients were on pressors when ischemia developed. Laboratory measures of coagulation, including platelet count, PT, PTT, fibrinogen, activated clotting time and heparin dose, did not show a clear trend in the days prior to development of ischemia. However, in several cases, there were increased transfusion requirements 24-48 hours before ischemia developed. Increased circuit clotting prompted a circuit change in two patients within 1-2 days of ischemia. In all patients, autopsy disclosed ischemic changes involving all four limbs; the penis and distal scrotum were involved in one patient. There were well-demarcated, confluent ecchymoses over the involved skin. A detailed examination of the limbs was permitted in one case. Large arteries were patent and normal proximally, with distal mural necrosis and hemorrhage. Involved tissues showed bland fibrin thrombi in the microcirculation, with tissue necrosis and hemorrhage.

Conclusion: We describe the first four cases of symmetrical peripheral gangrene complicating ECMO. The four pediatric patients were all status-post recent surgery for congenital cardiac disease, and all had significant ECMO exposure prior to developing limb ischemia. Laboratory tests of coagulation do not appear to predict this complication, but increased transfusion requirements may precede ischemia. Symmetrical peripheral gangrene is an unusual complication of ECMO that may arise in the setting of DIC, sepsis or other hemostatic imbalance.

45 Symmetrical Peripheral Gangrene in Four Children Receiving Extracorporeal Membrane Oxygenation Following Cardiac Surgery

RC Reed, Department of Pathology, Kosair Children's Hospital, Louisville, KY.

46 The Utility of Digital Slide Technology in Archiving Pediatric Consult Material: A Study of 50 Cases Submitted by Outside Institutions

A Agadi, C Belludi and K Stringer, Cincinnati Children's Hospital Medical Center; University of Cincinnati.

Background: Pathology consultation slides from outside institutions are received in the hundreds each year by many children's hospitals. The diversity of these submitted cases provides a rich substrate for trainee education, for research questions, and for nurturing the clinical acumen of the faculty members. Sadly this precious resource is often transient for the recipient institution because of requests to return original glass slides to originating sources. We asked if new digital slide technology, now commercially available, could help alleviate this shortcoming by providing a recipient institution with permanent "virtual slides" for subsequent retrieval using a computer.

Design: After determining the rate of return of consultation slides, representative slides were digitally scanned from each of 50 consecutive consultation cases received by our pediatric pathology department from outside hospitals. Records were kept of associated costs. The robotic instrument in our department used for scanning slides into computerized "virtual slides" was the Aperio company's new Scanscope XT, one of several state-of-the-art slide scanners now available from many companies worldwide. Slides were scanned using its faster, lower resolution settings and also its slower, higher resolution settings. During departmental slide conference, to an audience of faculty pathologists and pathology trainees who scored images, both of the resultant "virtual slides" were shown from selected cases. Using a five point scoring system they were compared to one another in terms of image quality, and also compared to the image from our microscope-projector pair (Nikon, Sony) usually used for that conference.

Results: An unfortunately high rate of return of 88 percent was determined for consultation slides, meaning the vast majority was going back to outside hospitals of origin. The 50 consecutive consult slides in this study were submitted from 17 states coast to coast and one overseas country. All viewers rated image quality as slightly better to markedly better for the higher resolution virtual slides compared to the images from the microscope-projector pair usually used in our conferences. The lower resolution virtual slides fared less well, nonetheless receiving from half of viewers an image quality rating higher than that of the microscope-projector pair. The cost to prepare a virtual slide, in terms of technician time and computer disc space, was comparable to preparing a stained recut (glass) slide, but the up-front purchase price of a machine to scan slides is higher than many machines to stain slides.

Conclusion: Digital slide technology offers a promising way of efficiently archiving consult slides within the recipient pediatric institution. In a cost effective manner it provides in perpetuity - using virtual microscopy software on departmental computers - a readily accessible collection of pediatric histopathology for subsequent visual examination and study.

47 Banking Placental Tissue: Optimization of Collection Procedures for Molecular Analysis

L Wolfe, V Tache, J Kim, WK Kwan, L Laurent, M Parast, University of California, San Diego, La Jolla, CA

Background: Banking of high-quality placental tissue specimens would be valuable for biomarker discovery and molecular studies. There is little data on standardized methodology for placental collection. In obstetrics, the time of delivery is unpredictable, making immediate tissue collection difficult. Our objective was to identify optimal timing and mode of collection for RNA and protein analysis.

Design: Two collection methods were used to obtain placental tissue over a period of 2 hours post-delivery (0, 30, 60, and 120 minutes). Method A used a traditional snap freeze technique in liquid nitrogen with storage at -80°C. Method B used a preservative (either RNA-Later, for RNA preservation, or All-protect Tissue Reagent/ATR, for preservation of protein) placed at 4°C for a minimum of 24 hours then transferred to -80°C. To assess a more convenient collection method, multiple small (4 mm thick) samples were collected in the preservative along with a larger (10 mm thick) sample, which was placed in the preservative at 4°C and later divided into small pieces. RNA was isolated using the miRvana kit; RNA quality was determined using RNA integrity number (RIN). Protein isolation was performed by placing samples in standard lysis buffer containing protease/phosphatase inhibitor cocktail, followed by homogenization with a beadbeater and sonicator. Total protein was quantified using the BCA assay. Western Blot analysis with markers of tissue stress and injury were performed, including phospho-MAP Kinase.

Results: RNA-Later-preserved tissue had higher and more consistent RINs compared to snap frozen tissue. Using an acceptable RIN value of 7.5, placental samples had an average acceptable RIN up to 60 minutes. Similar RINs were obtained for tissue collected in RNA-Later as large samples (average 7.3-8.0) compared to small samples (average 7.8-8.3). For protein, snap frozen samples collected at time 0 showed the lowest levels of tissue injury markers. However, tissues preserved in ATR, in either small or large pieces, showed significantly higher levels of stress markers, particularly of phosphorylated MAP Kinase. While variable between 30 and 90 minutes, most stress markers were significantly elevated at 120 minutes post-delivery.

Conclusion: RNA-Later preserves RNA quality and is superior to snap freezing for placental tissue. Using RNA-Later, high-quality RNA can be isolated from tissue collected up to 1 hour after delivery, and from a large sample that can be subdivided later. Traditional snap freezing is the best methodology for preservation of placental tissue for protein analysis. While early collection (by 30 minutes post-delivery) is required to minimize expression of some markers of cellular stress/injury, delayed collection (up to 90 minutes after delivery) may be acceptable for analysis of most pathways.

48 Duplication of the EWS Fusion Gene in Desmoplastic Small Round Cell Tumor, a Common FISH Finding.

KM Stashek, W Golden, EB Stelow, RD LeGallo, University of Virginia, Charlottesville, VA

Background: Desmoplastic small round cell tumor (DSRCT) is an aggressive neoplasm that typically occurs in the abdomen and pelvis of children and young adults. Like many soft tissue sarcomas, it harbors a unique translocation involving the Ewing sarcoma gene (EWS) on 22q12 and the Wilms' tumor gene (WT1) on 11p13 that creates a novel protein with oncogenic properties. Previous case reports have mentioned the complex cytogenetic abnormalities seen on karyotype, however little has been described about abnormalities seen utilizing FISH. In this study, we report six cases of DSRCT with variant signal patterns seen with FISH.

Design: Of 17 patients identified with desmoplastic small round cell tumor, six had corresponding FISH data. Results from interphase FISH using the VYSIS EWSR1 (22q12) dual color breakapart probe on tumor touch preps and monolayer cultures were evaluated. The intact gene is seen as a yellow signal, while a separate green and red signal separated by at least one signal width is considered to represent a disrupted gene.

Results: The six patients included 4 males and 2 females, who ranged in age from 6-33 years of age. Sites of tumor include abdomen (3 cases), pelvis (1 case), retroperitoneum (1 case) and periorbital soft tissue (1 case). Of the six cases, all showed complex genetic rearrangements involving the EWS fusion gene with five of the six showing duplication. The one case that showed only one disrupted gene showed loss of the intact counterpart. Three of these cases with duplication were confirmed using formalin fixed paraffin embedded (FFPE) tissue with identical signal patterns.

Results (table 1)

Age	Sex	Location	Signal Pattern	% cells with pattern
11	M	Retroperitoneum	2Y 2R 2G	73% (145/200)
26	F	Abdomen/ pelvis	1R 1G	92% (184/200)
6	F	Pelvis	2Y 2R 2G	72% (142/200)
33	M	Abdomen	1Y 2R 1G	59% (118/200)
			1Y 1R 1G	6.5% (13/200)
33	M	Abdomen	1Y 2R 2G	9.5% (19/200)
			2Y 2R 2G	79% (159/200)
18	M	Periorbital	2Y 2R 2G	51% (102/200)

Conclusion: As FISH has become increasingly utilized in the diagnosis of DSRCT, especially using FFPE tissue, it is important to recognize that variant signal patterns do occur, and may even be commonplace. Whether or not the duplication seen here represents low level amplification and affects pathogenesis or biologic behavior is uncertain, and needs to be further studied in a larger subset of patients.

49 TP53 Codon 72 Polymorphism in Favorable Histology Wilms Tumors

D Rakheja, NG Cost, S Khokhar, JA Wickiser, LA Baker, M Mitui, UT Southwestern Medical Center, Dallas, TX; Children's Medical Center, Dallas, TX.

Background: In Wilms tumors, TP53 mutations correlate with anaplastic morphology and chemoresistance, but the role of TP53 polymorphisms has not been studied in favorable histology Wilms tumors. Single nucleotide polymorphism at codon 72 of TP53 encoding either arginine (CGC) or proline (CCC) is suggested to result in altered biological and biochemical behavior of P53 in vitro. Compared to the proline (P) allele, the arginine (R) allele triggers a more pronounced apoptosis response, whereas the P allele induces more G1 arrest. In clinical studies, P allele has been associated with urothelial, thyroid, and colorectal carcinomas and chronic myeloid leukemia, P/P genotype with impaired response to chemoradiotherapy and reduced survival in head and neck carcinoma, R allele with reduction of survival in breast cancer patients, and R/R genotype with advanced lung cancer.

Design: We sequenced exons 2-4 and flanking intronic regions of TP53 in frozen tissue samples of 23 favorable histology Wilms tumors. The primer sequences for PCR and sequencing were previously published by Gonzalez et al., 2008. Sequencing reaction and capillary electrophoresis were performed at the institutional sequencing core and compared to NCBI reference sequence NM_000546.4.

Results: At the time of diagnosis, the 23 patients ranged in age from 8.3 to 99.6 months (mean 39.2, median 34.5). The National Wilms Tumor Study Group (NWTS) stage was I (2 patients), II (10 patients), III (5 patients), IV (5 patients), and V (1 patient). Two patients had recurrent disease over a follow-up period that ranged from 1.8 months to 68.2 months (mean 29, median 29.4). The genotype frequencies were: R/R 0.61, P/R 0.35, P/P 0.04 and the allele frequencies were R 0.78 and P 0.22. The genotype and allele frequencies showed no significant correlation with age at diagnosis, NWTS stage, or development of recurrence.

Conclusion: Our cohort of Wilms tumors showed an over-representation of the R allele and the R/R genotype. According to the National Cancer Institute's SNP500Cancer database, R allele frequency in the general population is 0.44-0.46 and genotype frequencies are R/R 0.24-0.27, P/R 0.36-0.40, P/P 0.34-0.36, compared to R allele frequency of 0.78 and the R/R genotype frequency of 0.61 in our cohort. While there was no significant correlation of TP53 codon 72 polymorphism with age at diagnosis, NWTS stage, or development of recurrence in Wilms tumors, our study is limited by small sample size and short follow-up.

50 Correlation Between XIAP Protein Expression and Risk of Bone Marrow Metastasis in Children With Neuroblastoma

Jailan Hanafy, *Susana Galli MD, Ali G. Saad MD, Jailan M. Osman MD, Arkansas Children's Hospital, and *Laboratory of Pathology, National Cancer Institute, National Institute of Health, Bethesda, Maryland.

Background: Neuroblastoma (NB) is a common pediatric neoplasm. Histopathological features remain a poor predictor of the risk of developing distant metastases, in particular bone marrow (BM) metastasis, in patients with NB. Despite significant advances in therapeutic protocols, the prognosis of children with NB remains largely poor. XIAP (X chromosome-linked inhibitor of apoptosis protein) belongs to the family of IAPs (inhibitor of apoptosis proteins) and has been shown to inhibit apoptosis induced by various stimuli, including chemotherapeutic agents, in different cell types. We conducted this study to investigate the correlation between the expressions of XIAP (anti-apoptotic) protein and the development of BM metastasis.

Design: Twelve patients (group 1) with NB and negative BM and 7 patients (group 2) with NB and positive BM are included in this study. Samples from the primary tumor are immunostained with antibodies against XIAP. Immunostaining was evaluated by 2 pathologists. The percentage of XIAP positive tumor cells was estimated using semi-quantitative method. The expression of each XIAP is correlated with the development of BM metastasis.

Results: Group 1 (mean age 20.6 months; range 0.5-61 months) consisted of 7 males and 5 females. Group 2 (mean age 67.8 months; range 24-156 months) consisted of 3 males and 4 females. There was a statistical difference in the expression of XIAP between both groups (P=0.02). There was no expression of XIAP in one patient with bone marrow metastasis. The reason for this finding is unclear at the moment. Details of the expression of XIAP is summarized in Table 1:

Percentage of XIAP expression in neuroblastoma tumor cells

Neuroblastoma	XIAP	
	Mean	Range
With BM metastasis	59%	40-90
Without BM metastasis	87%	70-100

Conclusion: Our data indicate that XIAP is widely expressed in NB with and without bone metastasis. Furthermore, our study demonstrates that BM metastasis was found in patients with low expression of XIAP significantly and therefore more extensive studies will help address its potential implication in patient outcome.

51 Correlation Between Nestin Expression and Risk of Bone Marrow Metastasis in Children With Neuroblastoma

Mohammad T. Hanafy, *Susana Galli MD., Jailan M. Osman MD, Arkansas Children's Hospital, Little Rock, Arkansas and *Laboratory of Pathology, National Cancer Institute, National Institute of Health, Bethesda, Maryland.

Background: Neuroblastoma (NB) is a common pediatric neoplasm. Histopathological features remain a poor predictor of the risk of developing distant

metastases, in particular bone marrow (BM) metastasis, in patients with NB. Nestin, as an intermediate filament (IF) protein, is expressed in proliferating progenitor cells of developmental and regenerating tissues, and is identified as a neuroepithelial precursor cell marker. Recently, nestin was detected in many tumors. Moreover, the expression intensity of nestin exhibited significant correlation with the malignant grade of different neoplasm. In this study we aim to evaluate and correlate nestin expression in NB patients with and without BM metastasis.

Design: Twelve patients (group 1) with NB and negative BM and 7 patients (group 2) with NB and positive BM are included in this study. Samples from the primary tumor are immunostained with antibodies against nestin. Immunostaining was evaluated by 2 pathologists. The percentage of nestin positive tumor cells was estimated using semi-quantitative method.

Results: Group 1 (mean age 20.6 months; range 0.5-61 months) consisted of 7 males and 5 females. Group 2 (mean age 67.8 months; range 24-156 months) consisted of 3 males and 4 females. Nestin was expressed in group 1 (11/12) and in group 2 (5/7). We observed no significant difference between the expression of nestin in NB patient with BM metastasis and that without BM metastasis (p=0.4). Furthermore, there was no expression of nestin in one patient with bone marrow metastasis, and in two patients without BM metastasis. The reason for this finding will be further studied. Details of the expression of Nestin is summarized in Table 1:

Percentage of Nestin expression in neuroblastoma tumor cells

Neuroblastoma	Nestin	
	Mean	Range
With BM metastasis	76%	60-90
Without BM metastasis	77%	30-100

Conclusion: The data show that expression of nestin in neuroblastoma was detected regardless of the degree of differentiation. In addition, the consistent expression of nestin in primary neuroblastoma tumors does not correlate with bone marrow metastasis.

SPP Author Index

Abramowsky, CR	3, 32, 34, 35	Galli, S	50, 51	Litten, JB	8	Shehata, BM	3, 32, 34, 35
Agadi, A	46	Gamis, AS	20, 21	London, WB	4	Shimada, H	4
Alexandrescu, S	6	Gastier-Foster, JM	4	Look, AT	4	Shulman, SC	3, 32, 34, 35
Alonzo, TA	20, 21	Gerbing, RB	20, 21	Lopez-Terrada, DH	24	Siebert, JR	10
Amatruda, JF	7, 23	Glass, IA	10	Lossia, A	13	Smith, FO	20, 21
Aplenc, R	20, 21	Glick, K	20, 21	Lotan, Y	23	Smith, KJ	10
Baker, LA	49	Golden, W	48	Machut, K	13	Sredni, ST	17
Bakken, P	37	Gross-Weissmann, ML	9	Macpherson, T	14	Stanek, J	41
Basu, D	26, 27	Gupta, A	28	Madan-Khetarpal, S	14	Stashek, KM	48
Belludi, C	46	Gwin, K	11, 22	Mahle, WT	35	Steinmetz, J	11, 22
Bouzyk, M	3, 32, 34	Han, J	19	Margraf, LR	2	Stelow, EB	48
Bowers, DC	2	Hanafy, J	50	Maris, JM	4	Stockman, DL	44
Brown, RE	6	Hanafy, M	51	McGranahan, A	43	Stringer, K	46
Brugarolas, J	2, 23	He, M	40	Mehta, V	24	Suganuma, R	4
Buell-Gutbrod, R	11	Heerema-McKenney, A	20, 21, 25	Melish, M	36	Sung, CJ	40
Buell-Gutbrod, R	22	Heider, A	26	Meshinchi, S	20, 21	Sung, L	20, 21
Burden, L	20, 21	Hill, DA	18	Mitui, M	49	Surti, U	14
Burns, N	6	Hirsch, BA	20, 21	Montag, A	11, 22	Tache, V	47
Byron, P	20, 21	Hoffman, I	28	Naranjo, A	4	Tang, W	3, 32, 34
Cajaiba, MM	14, 42	Hogarty, MD	4	Oble, DA	12	Tatevian, N	6
Calicchio, ML	38	Hogarty, MD	4	Osman, JM	50, 51	Terry, J	38
Castro, EC	15, 16	Hoover, JM	14	Parast, MM	33	Thompson, MP	31
Castro-Silva, F	43	Horton, CJ	2	Parast, M	47	Thompson, KS	36
Chang, KTE	39	Huang, MH	13	Park, JR	4	Timmons, CF	19
Chang, KW	25	Hwang, WS	39	Parks, TW	16	Tomlinson, GE	8
Chen, TT	8	Jarzembowski, JA	18, 44	Piecha, G	9	Tovar, JP	4
Chisholm, KM	20, 21, 25	Johnson-Welch, S	31	Pinar, H	40	Trivedi, N	33
Chou, PM	17	Kahwash, S	20, 21, 42	Plunkett, BP	13	Truong, MT	25
Cleveland, DS	29	Kapur, R	5	Prieto, VG	24	Turner, M	37
Cohn, SL	4	Kapur, P	23	Punia, JN	24, 30	Wallas, T	20, 21
Cole, B	5	Katzenstein, HM	3, 32, 34	Qin, X	5	Wang, WL	24
Cope-Yokoyama, S	29	Katzman, PJ	12	Raimondi, SC	20, 21	Wang, LL	4
Cost, NG	49	Kearney, DL	36	Raisanen, J	1	Warren, M	36
Covinsky, M	6	Khokhar, S	7, 8, 19, 23, 49	Rakheja, D	1, 2, 7, 8, 19, 23, 49	Webb, CR	30
Cox, T	10	Kim, J	47	Rebbaa, A	26, 27	Weng, J	40
Czerniak, BA	6	Koleganova, N	9	Rector, AD	30	West, RB	25
David, F	3, 32, 34	Kwan, WK	47	Reed, RC	45	Wickiser, JA	49
Davies, SM	20, 21	Kwok, S	25	Revell, PA	30	Winter, L	20, 21
Davis, A	26	Lacoursiere, Y	33	Reyes-Mugica, M	26, 27	Witchel, S	14
Dehner, LP	18	Langess, S	3, 32, 34	Ritz, E	9	Wolfe, L	47
Deisch, J	1	Laurent, L	47	Rosales, C	24	Woods, CM	28
Deregnier, R	13	Lazar, AJ	24	Rossi, MR	3, 32, 34	Zambrano, EV	44
Drehner, D	37	LeGallo, RD	48	Ryan, WD	33	Zhang, M	5
Ernst, LM	13	Liang, X	43	Saad, AG	50		
Fustino, N	7, 23	Lin, J	5	Sacks, JH	35		
Galambos, C	15, 16			Schultz, R	8		
				Shao, J	43		