

but not in the keratinized superficial layers; the pattern for  $\beta$ I spectrin was the inverse for  $\alpha$ II spectrin. For example, we found  $\beta$ I spectrin in the superficial, but not in the basal epithelium of the uterine cervix. Compared to their normal epithelial counterparts, there was loss of  $\alpha$ II spectrin in papillary thyroid carcinoma, in clear cell renal carcinoma and in invasive ductal breast carcinoma. Expression of  $\beta$ I spectrin was limited to normal hepatocytes, transitional umbrella cells of the bladder, type II pneumocytes of the lung, mature superficial squamous epithelium of the uterine cervix, and lobular and ductal epithelium of the breast. In contrast to the absence of staining for  $\beta$ I spectrin in normal biliary ductules, thyroid follicles and renal tubules, we detected aberrant expression of  $\beta$ I spectrin in biliary ductal carcinoma, thyroid papillary carcinoma and renal clear cell carcinoma. Spectrin isoforms  $\beta$ II and  $\beta$ III were universally present in both benign and malignant epithelial tumors.

**Conclusions:** Loss or gain of specific spectrin isoforms occur both during normal epithelial maturation and in some examples of neoplastic transformation. This suggests the possibility of a significant role for spectrin isoforms in carcinogenesis.

#### 1752 MDM2 Amplification in Atypical Lipomatous Tumors/Well-Differentiated Liposarcomas and Dedifferentiated Liposarcomas: A Comparative RT-PCR and FISH Study

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**Background:** As future therapeutic options for well-differentiated and dedifferentiated liposarcomas are likely to include targeting the MDM2 pathway, reliable methods for its demonstration will be required. A comparative study of the sensitivity of RT-PCR versus FISH for demonstrating amplification of MDM2 in such tumors was therefore performed.

**Design:** 30 consecutive resected atypical lipomatous tumors/well-differentiated liposarcomas and dedifferentiated liposarcomas as well as 6 control adipocytic tumors of different type, were reviewed by two pathologists and subsequently investigated for amplification of MDM2. Genomic DNA was extracted from formalin fixed paraffin embedded tissues using the Qiagen Qia Amp FFPE kit. Fifty nanogram of each FFPE DNA was used in a multiplex real-time PCR reaction using the FAM (MDM2) and VIC (RNase P as internal control) labeled probes on an ABI prism 7000. To distinguish samples with high MDM2 copy number from those with normal copy number, the standard delta-Ct method was used. Neoplasms with high copy number were judged to be amplified. FISH dual copy assay was performed using Poseidon Repeat Free MDM2 (12q15) & SE12 control probe. More than 4 signals were considered evidence of amplification.

**Results:** 1-On FISH analysis 29 of 30 atypical lipomatous tumors/well-differentiated liposarcomas and dedifferentiated liposarcomas were found to be amplified for MDM2, whereas 1 case of dedifferentiated liposarcoma was not. 2- On RT-PCR analysis of the same 30 tumors, 28 showed MDM2 amplification. Unamplified were the above mentioned case of dedifferentiated liposarcoma and 1 case of well-differentiated liposarcoma of inflammatory type. 3- None of the adipocytic tumors of other type showed MDM2 amplification by either method.

**Conclusions:** In atypical lipomatous tumors/well-differentiated liposarcomas and dedifferentiated liposarcomas demonstration of MDM2 amplification as measured by FISH was 96.6% and by RT-PCR 93.3%. Following methodological adjustments results could be improved.

#### 1753 15-Lipoxygenase 1 Promotes Hypoxia-Inducible Factor 1 $\alpha$ Degradation Dependent of Essential Enzymatic Function

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**Background:** Hypoxia inducible factor-1 $\alpha$  (HIF-1 $\alpha$ ) is the major subunit of HIF-1, critical in transcriptional regulation of genes whose products mediate angiogenesis and hypoxic adaptation. HIF-1 $\alpha$  is overexpressed in human cancers, predominately through defects in degradation pathway. Mammalian lipoxygenases (LOXs) are key mediators of arachidonic acid metabolism relevant to carcinogenesis. 15-LOX-1 has recently been demonstrated to be down-regulated in several common malignant tumors, and the degree of its down-regulation is reversely correlated to advanced tumor stages. Our prior studies have shown that inhibiting 15-LOX-1 increases HIF-1 $\alpha$  level and HIF-1 transcriptional activity (2002 AACR) while mechanistic details remain elusive.

**Design:** Prostate cancer and colon cancer cell lines and human tissue microarray were employed. 15-LOX-1 expression vector was subcloned so as to manipulate its enzymatic activity. Stable or transient transfection plus luciferase assays, immunoprecipitation, HIF-1 $\alpha$  ubiquitination assay, and immunohistochemistry were utilized in the study.

**Results:** Here, we report that 15-LOX-1 promotes HIF-1 $\alpha$  ubiquitination and degradation, depending on the classic Pro<sup>64</sup>/hydroxylation/26S proteasome system under normoxia. Both enzymatic activity and intracellular membrane binding function of 15-LOX-1 are required to degrade HIF-1 $\alpha$ . The enhanced HIF-1 $\alpha$  degradation by 15-LOX-1 overexpression is not abolished even if proteasomal degradation is inhibited by hypoxia or CoCl<sub>2</sub>. No physical association between immunoprecipitated HIF-1 $\alpha$  and 15-LOX-1 occurs, which further supports that enzymatic products of 15-LOX-1 likely mediate the decrease in HIF-1 $\alpha$ . In addition, induction of endogenous 15-LOX-1 simultaneously reduces hypoxia-induced HIF-1 $\alpha$  accumulation in a dose dependent pattern. Finally, 15-LOX-1 down-regulation was demonstrated by immunohistochemical studies in breast or other common malignancies compared to their benign counterparts.

**Conclusions:** Our findings provide a novel mechanism for the HIF-1 $\alpha$  regulation that combines oxygen dependent and oxygen insensitive actions. This is the first evidence demonstrating that a lipoxygenase involves HIF-1 $\alpha$  regulation. Lowered 15-LOX-1 is probably one of the many reasons of HIF-1 $\alpha$  overexpression in human cancers, which contributes to diseases progression.

#### 1754 Significance of Loss of Heterozygosities in Predicting Sentinel Lymph Node Metastasis of Breast Invasive Ductal Carcinoma

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**Background:** Sentinel lymph node (SLN) metastasis of breast invasive ductal cancer (BIDC) is one of the main criteria determining stage that is the strongest predictor of disease aggressiveness and survival, and determining the need for axillary resection and additional therapy. We studied SLN metastasis using a combined histopathologic/molecular approach to gain insights into the pathobiology implications.

**Design:** Fourteen BIDC patients with positive SLN and 19 with negative SLN were retrieved. Analysis of 17 polymorphic microsatellite repeat markers targeting 1p34-36, 3p24-26, 5q23, 9p21, 10q23, 17p13, 17q12, 17q21, 21q22, and 22q13 was performed in DNA isolated from primary tumors and metastatic tumors.

**Results:** BIDCs with SLN metastasis showed larger size (p=0.004) and higher nuclear grade (P=0.047) than those with negative SLN, which correlated with higher fractional mutation rate (FMR) of the primary tumor and shared FMR by the primary and SLN tumors. SLN metastasis was not related with expression of estrogen receptor, progesterone receptor, and Her2/neu, and did not correlate with higher FMR. However, higher FMR was related to higher percentage of positive SLNs. Tumor size is not necessarily a strong marker for the prediction of aggressiveness and metastasis of BIDC, indicating that some smaller BIDCs arise intrinsically as an aggressive malignancy with metastatic disease. LOHs at 1p34-36, 3p24-26, 9p21, 10q23, 17p13, 17q12, 21q22 and 22q13 may play an important role in the development and aggressiveness of BIDC. LOHs at 1p34-36, 17p13 and 22q13 may play an important role in metastasis. None of the LOHs were shared by all the tumors, suggesting that BIDC develops using various pathways that have unique and personalized patterns of mutational changes.

**Conclusions:** Detection of LOH is useful in studying oncogenesis and predicting aggressiveness and metastasis of BIDC.

## Pediatrics

#### 1755 Morphoproteomic Profiling of Choroid Plexus Carcinoma Reveals Constitutive Activation of the mTOR and ERK Pathways with Cell Cycle Progression

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**Background:** Choroid plexus carcinoma (CPC) is a rare pediatric malignancy with a poor prognosis despite conventional therapies. Understanding its molecular biology should provide insight into targeted therapeutic options. To this end, we performed morphoproteomic profiling on two such cases.

**Design:** Sections of an original and recurrent CPC, were available for analysis. Immunohistochemical probes were applied to detect the following: 1. phosphorylated (p)-mammalian target of rapamycin (mTOR) [Ser 2448]; p-Akt (Ser 473); p-p70S6K (Thr 389); and p-extracellular signal-regulated kinase (ERK) 1/2 (Thr 202/Tyr 204); and 2. cell cycle-related proteins to include nuclear Ki-67(G1, S, G2 and M phases), S-phase kinase-associated protein (Skp2) (promotes G1 to S), p53 (wild type regulates, mutant form permissive in G1 to S) and topoisomerase II alpha (facilitates S to G2/M). Quantification of the nuclear percentages was carried out, whenever possible, by an automated cellular imaging system (ACIS). Mitotic indices were calculated as an average of mitotic figures in the most mitotically active regions per sets of ten high power fields (h.p.f.'s).

**Results:** Constitutive activation of the Akt/mTOR and ras/Raf kinase/ERK pathways is evident in both cases with predominantly plasmalemmal and cytoplasmic expressions of p-Akt (Ser 473) and p-mTOR (Ser 2448), favoring the mTORC1 pathway, and with nuclear expression of p-p70S6K (Thr 389) and nuclear p-ERK 1/2 (Thr 202/Tyr 204). Correlative cell cycle progression was noted with mean nuclear expressions in each of the cases as follows: Ki-67 at 14.0 and 11.6%, Skp2 at ~20% (visual quantification) and 7.8% and 28 and 27 mitotic figures per 10 h.p.f.'s, respectively. Nuclear topoisomerase II alpha at 96 and 52.1% and p53 at ~30 and 63% (probably mutant form) were noted.

**Conclusions:** Morphoproteomic profiling reveals constitutive activation of the Akt/mTOR and ras/Raf kinase/ERK pathways, which appears to be the first report of its kind in CPC. Such activation coincides with downstream signal transduction from insulin-like growth factor (IGF) II/IGF-type 1 receptor and platelet-derived growth factor receptor, previously reported in CPC. Moreover, it accords with the cell cycle progression and provides opportunities to apply combinatorial therapies to target the mTORC1 pathway, cell cycle progression and topoisomerase II alpha in CPC.

#### 1756 Sorafenib Down Regulates Akt and STAT3 Survival Pathways and Induces Apoptosis in a Human Neuroblastoma Cell Line

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**Background:** Neuroblastoma ranks second among solid tumors in children, and its tumorigenicity is enhanced by the expression of survival pathways such as Akt, Nuclear Factor (NF)-kappaB and signal transducer and activator of transcription (STAT)3 and the expression of the anti-apoptotic protein, Mcl-1. Sorafenib is a multi-kinase inhibitor that also inhibits STAT3 signaling and induces apoptosis in association with downregulation of Mcl-1. In this study, we examine the efficacy of sorafenib on a human neuroblastoma cell line and also investigate its possible mechanisms.

**Design:** A neuroblastoma cell line, SK-N-AS, was purchased from ATCC. After reaching 50-60% confluence, cells were treated with various concentrations of sorafenib (0, 0.1, 1, 5, 10 and 20  $\mu$ M) for different period of times (2, 6, 24, 48 and 72 hours). The cell viability was determined by MTT colorimetric assay. The apoptotic responses were

measured with TUNEL assay. Phosphorylation of Akt1/2/3 (pAkt1/2/3[Thr308]), AMP-activated protein kinase alpha1 subunit (p-AMPK $\alpha$ 1[Thr172]), STAT3 (p-STAT3[Ser727]), and phospholipase D2 (PLD2) protein expression levels were determined with Western blot assay.

**Results:** As early as 2 hours post-treatment, cell viability was significantly decreased at 10  $\mu$ M concentration. In 24 hours or longer treatment groups, sorafenib at 5  $\mu$ M and above concentrations significantly decreased cell viability. TUNEL assay showed similar results. A significant increase of apoptosis was observed in 5 and 20  $\mu$ M treatment groups 24 hours after treatment. Western blots showed a decrease of p-Akt1/2/3 and p-STAT3 expression levels in sorafenib treatment groups. A slight increase of p-AMPK $\alpha$ 1 was also observed. PLD2 levels remained unchanged.

**Conclusions:** Our results indicate that sorafenib significantly decreased cell viability and increased apoptosis in a human neuroblastoma cell line in association with down regulation of p-Akt and p-STAT3 survival pathways. These data suggested a potential clinical application of sorafenib in the treatment of neuroblastoma cases with constitutive activation of Akt and STAT3.

### 1757 Gross Umbilical Cord Abnormalities: Histologic Sequelae and Association with Hypoxia

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**Background:** Umbilical cord complications (CC) such as true knots (TK), velamentous insertion (VEL), marginal insertion (MUC), entanglement (CE), excessive cord length (ELUC) or excessive coiling (ETUC) can lead to decreased umbilical blood flow and have been associated with adverse outcome and demise (IUFD). Few large series exist correlating CC with placental pathology. We present the largest series of CC at this time.

**Design:** Search of our database from 1998-2008 for 3rd trimester placentas with TK, VEL, MUC, CE, ELUC, or ETUC identified 840 cases. 200 gestational age matched controls were selected randomly. Lesions associated with circulatory stasis and thrombosis (CST) including villous congestion (VC), umbilical vessel distension (UVD), chorionic plate vessel distension (CPD), umbilical vessel thrombosis (UVT), fetal vascular thrombosis (FVT), villous stromal karyorrhexis (VSK) and avascular villi (AV) were scored as well as any other pathologic lesions. Data was analyzed by ANOVA and Chi square, with  $p < .05$  statistically significant.

**Results:** CC as a group was associated with a significant increase in VC ( $p = .039$ ), UVD ( $p = .005$ ), UVT ( $p < .001$ ), FVT ( $p < .001$ ), and AV ( $p = .002$ ). TK or ETUC alone showed significant increases in these lesions (all  $p < 0.05$ ). VUC, CE, or ELUC showed an increase for some but not all lesions ( $p < 0.05$ ). MUC was not associated with any lesion. Lesions associated with hypoxia, nucleated red blood cells (RBC) and chorangiomas (CHS), were increased in most groups and CC overall. Increased perivillous fibrin (IPVF) was increased in all but the TK group. CPD and VSK were not associated with any CC. Finally, the presence of any CC was significantly associated with IUFD ( $p = .002$ ).

**Conclusions:** First, similar to previous smaller series, we found a significant correlation between CC and CST lesions. Second, unlike prior studies, UVD and VC were associated with CC, but not CPD. VC may be a superior indicator of venous stasis along with UPD when compared to CPD. There may also be differences in definition or specificity of these lesions. Third, MUC has often been included with CC but our data indicate it is not associated with sequelae of CC. Finally, RBC, CHS, and IPVF were associated with CC which has not been previously described. As RBC and CHS are associated with hypoxia, this suggests that CC lead to intrauterine hypoxia and subsequent adverse outcome. IPVF may develop due to villous damage from CST or a tendency for coagulation; an association requiring further elucidation.

### 1758 Desmoid Fibromatosis (DES) in Childhood and Adolescence: An Analysis of 65 Patients in the First Two Decades of Life

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**Background:** DES is an intermediate soft tissue neoplasm that recurs, can be associated with APC mutation, and may be preceded by Gardner fibroma (GAF). We investigated clinicopathologic features, associated conditions, and outcome in young patients.

**Design:** 65 patients newborn to 20 years with DES were identified from surgical pathology and consultation files (7 reported previously, AJSP, 2007). Pathology materials and medical records were reviewed. Immunohistochemistry for beta-catenin was performed.

**Results:** 65 patients ranged from 2 months to 20 years at first diagnosis of DES; they had 74 separate DES, including 8 with multifocal DES, 13 with one or more GAF. There were 32 males and 33 females. At first diagnosis of DES, 3 (4%) were less than 1 year old, 22 (34%) were 1-9 years old, and 40 (62%) were 10-20 years old. Among the 74 DES, 36 (49%) arose on the trunk, 26 (35%) on the extremities, and 12 (16%) in the head and neck. Tumor diameter ranged from 2 to 25 cm. The circumscribed masses had a fleshy, whorled, white-tan cut surface. Sweeping fascicles of spindle cells displayed low to moderate cellularity, focal myxoid change, focal prominent collagen, a sparse mast cell infiltrate, and elongated, thin-walled perivascular blood vessels. Nuclei had fine chromatin, without atypia or atypical mitoses. Variations included prominent stellate myofibroblasts, hypercellularity, peripheral lymphoid aggregates, and contiguous GAF. 87% had nuclear beta-catenin positivity. All were treated with surgery; 5 also had chemotherapy. Followup in 24 patients revealed 13 with local recurrences (rate 54%), 6 with known APC, 1 with previous colon polyp, 1 with tuberous sclerosis, 1 with previous liver transplant. None had died of DES at last followup.

**Conclusions:** This large series of DES in young patients confirms its occurrence throughout childhood and especially in adolescence, documents an association with GAF and APC, reveals a recurrence rate of 54%, and confirms a predilection for the extremities and trunk, including the abdomen, mesentery, and pelvis. Among the patients with recurrent DES, 62% had GAF and/or APC and/or colon polyps. These observations emphasize the risk of recurrence, multifocality, and potential for associated APC in young

patients with DES. The frequency of APC in this series is very likely to be underestimated due to incomplete clinical information and family history. Children and adolescents with DES could benefit from clinical evaluation for potential APC.

### 1759 ETV6 Gene Rearrangement in Soft Tissue and Renal Spindle Cell Tumors of Infants and Neonates: Correlation with Morphology and Clinical Follow-Up Data

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**Background:** ETV6 gene rearrangement as a result of t(12;15)(p13;q26) is a recurrent chromosomal abnormality in infantile fibrosarcoma (IFS) and cellular congenital mesoblastic nephroma (CMN). Differential diagnosis of the two entities that includes their benign counterparts is sometimes difficult. The role of fluorescent in situ hybridization (FISH) in the differential diagnosis and prognosis of these lesions has not been studied systematically.

**Design:** We analyzed 27 tumors with the original diagnoses of IFS (n=13), CMN (n=8), infantile hemangiopericytoma (IHP, n=4) and infantile myofibromatosis (IMF, n=2) from the files of our institution. Morphology was blindly re-reviewed by 2 pathologists; current IHC panel for spindle cell tumors with appropriate controls was applied to difficult cases. Interphase FISH with dual color split apart ETV6 probe (DakoCytomation, Denmark) was performed on FFPE sections of the archival material. The treatment and follow up data available for 22 of 27 patients were analyzed in accord with the NIH guidelines on human subject research with an appropriate IRB approval.

**Results:** Pathology review reclassified 6 of the 27 tumors and revised diagnoses included 10 IFS, 5 cellular CMN, 2 classical CMN, 1 mixed CMN, 3 IHP, 3 IMF, 1 plexiform fibro-histiocytic tumor, 1 malignant peripheral nerve sheath tumor (MPNST) and 1 undifferentiated sarcoma. The ETV6 was re-arranged in 9 of 10 IFS, 2 of 5 cellular CMN, none of 3 classical and mixed CMN, 3 IHP and 3 IMF. Remaining reclassified tumors showed no rearrangement; however, hybridization signals consistent with trisomy 12 were observed in the MPNST as well as in the rearrangement-negative IFS. All IFSs, 3 CMNs, 2 IHPs and 1 IMF were treated with chemotherapy and definitive surgery; 5 CMNs, 1 HPC and 2 IMF - surgery only. All but 1 (MPNST) patients are alive with no evidence of disease in 1-34 yr (mean 11.4 yr, median 9 yr).

**Conclusions:** Interphase FISH for the ETV6 rearrangement is helpful in differentiating IFS from its mimics and can be routinely used on FFPE. Cellular CMN showed rearrangement in less than half of the cases and the ETV6-rearrangement positive cellular CMNs were cured by surgery only even when presented at stage III. Regardless of the rearrangement status both soft tissue and renal infantile fibrous tumors appear to have an excellent prognosis with current treatment modalities.

### 1760 Immunohistochemical Expression of Cytokeratin 20 in Molar Gestation

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**Background:** Hydatidiform moles are gestational trophoblastic diseases with a potential for aggressive behavior. The differential diagnosis between complete hydatidiform moles (CHM), partial hydatidiform moles (PHM) and hydropic gestations (HG) can be challenging on morphological basis and the use of ancillary diagnostics is often necessary. Recent studies have suggested that cytokeratin 20 (CK20) is significantly overexpressed in molar gestations and could be used as a diagnostic tool. The aim of this study is to analyze the expression of CK20 in CHM, PHM, HG and normal products of conception (POC) on a large cohort of well documented cases. We also evaluated if CK20 was associated with aggressive behavior (persistence, invasion or malignant transformation) in CHM.

**Design:** The study cohort consists of 87 consecutive cases of CHM (n=58) and PHM (n=29) accessioned in our department between 1993 and 2008. Cases of HG (n=28) and POC (n=16) were used as a control group. All slides were reviewed and in most cases the diagnosis was supported by ancillary techniques (staining with p57kip2, flow cytometry, and/or in situ hybridization). All patients were treated primarily by curettage or aspiration with or without adjuvant chemotherapy. Follow up information was retrieved. Sections were stained with CK20 and immunostaining was recorded as positive only when strong staining was present in >25% of trophoblastic cells.

**Results:** Staining for CK20 was positive in 9/58 CHM, 0/29 PHM, 1/28 HG, 2/16 POC. Follow-up was available in 46/58 CHM (mean follow-up: 14 months). When CHM are compared to all other diagnoses, positivity for CK20 has a sensitivity of 15.5%, a specificity of 95.9% and a total accuracy of 74% (p value=0.025, chi-square test). 15 CHM had an aggressive behavior (12 persistent, 1 invasive and 2 metastatic moles) and were all negative for CK20. Follow-up was available in 5/9 CK20 positive CHM which all had a favorable outcome (Table below).

	Benign	Aggressive	Total
CK20+	5	0	5 CHM
CK20-	26	15	41 CHM
Total	31	15	46 CHM

**Conclusions:** Our IHC results confirm that CK20 is expressed in some CHM. However, contrary to previous reports, CK20 was expressed in only a small proportion of CHM (15.5%), it was not expressed in PHM and it was also expressed in few cases of HG and POC. Even though CK20 has a limited value as a diagnostic tool, CK20 positivity can support a diagnosis of CHM. Interestingly, CK20 positivity in CHM appears to be an indicator of favorable outcome.

### 1761 Nestin Expression in Ewing Sarcoma Family Tumors

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**Background:** Nestin is an intermediate filament protein, which is expressed in pluripotent embryonic stem cells. It has been detected in several tumors, especially



in those of neuroectodermal and mesenchymal origin. ESFT are characterized by specific translocations, the most common of which is EWS/FLI1. Recent data have pointed to a mesenchymal stem cell phenotype of ESFT cells with silenced EWS/FLI1. Because nestin is a marker of progenitor/stem cells, we addressed whether EWS/FLI1 downregulation induces nestin expression in ESFT cells *in vitro* and explored the extent of nestin expression in ESFT tissues and cell lines.

**Design:** We examined the expression of nestin by immunohistochemistry in 55 formalin-fixed paraffin-embedded tumor tissue samples from 38 patients treated in our institution. In 8 patients, several tissue samples (2 to 4 per patient) obtained over a period of 2 to 17 years were available for review. We also analyzed nestin protein expression by Western blot in 14 ESFT cell lines, and in 2 cell clones of the TC-71 ESFT cell line that had been stably transfected with EWS/FLI1 short hairpin (sh) RNA plasmid to silence EWS/FLI1. The presence of EWS/FLI1 was evaluated by RT-PCR in all cell lines.

**Results:** From the 38 patients, only 2 had nestin-positive tumor samples at diagnosis with 1% to 5% positive cells correspondingly. One of these patients developed a higher percentage (20% and 40%) of nestin-positive cells in two subsequent biopsies. Another patient whose original tumor was negative for nestin showed expression in subsequent biopsies (40%). ESFT cell lines were more frequently positive for nestin (9/14 positive). Highest expression was found in one cell line with a variant EWS/FLI1 fusion transcript and in the 2 clones with silenced EWS/FLI1.

**Conclusions:** Our data show that: 1) nestin is minimally expressed in ESFT tissue at diagnosis, but can be induced in some patients 2) increased nestin expression correlates with EWS/FLI1 downregulation or presence of EWS/FLI1 variant. Because relapses in ESFT have been attributed to stem cells with undetectable EWS/FLI1 (dormant cells), nestin may be used as a marker to detect stem cells in ESFT and may play a role in the biology of the tumor.

**1762 Renal Tumors in Children beyond the Age of 10 Years**

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**Background:** Wilms tumor (WT) is the most common renal tumor in children, but, in addition, different epithelial, mesenchymal, neuroectodermal and hematopoietic neoplasms may also arise in the kidney during childhood. Several of these tumors show specific age distribution: in the first year of life mesoblastic nephroma and rhabdoid tumor are more common than in older children, whereas renal cell carcinoma (RCC), primitive neuroectodermal tumor (PNET) and anaplastic WT rarely occur in infants. The aim of this study is to demonstrate a spectrum of renal tumors in children aged 10 to 16 years.

**Design:** For this study we used data from 1492 cases from UKW3 (1991-2001) and SIOP (2002-2008) clinical trials. All cases were submitted for central pathology review and cases from the UKW3 trial were reclassified according to the current criteria.

**Results:** In total 69/1492 (4.6%) tumors in children aged 10 to 16 years were identified including 50 WTs (73.5% of all renal tumours in this group), 10 RCC (14.7%), three (4.3%) renal medullary carcinoma, two (3%) PNET; one (1.4%) clear cell sarcoma of kidney, one desmoplastic small round cell tumor, and one case where the diagnosis of a malignant tumor of uncertain origin was made. The frequency of WT with diffuse anaplasia in this age group was rather high – 7/69 (10%) including 9.4% in the SIOP and 14.9% in UKW3 trials. This contrasts with the frequency of WT with diffuse anaplasia in patients under 10 years of age - 5.5% and 4%. In the group of RCC, the following types were identified: RCC tXp 11.2 - 4 cases; papillary type II - 3 cases; papillary RCC type I - 1 case; clear cell RCC - 1, RCC unclassified - 1 case.

**Conclusions:** Our result showed that among renal tumours in children 10 - 16 years of age WT is still the most common malignancy although showing significantly higher proportion of diffuse anaplasia. Second most common type of tumors were different variants of RCC and their frequency is much higher than in younger patients.

**1763 Pediatric Patients with Non-Specific Abdominal Pain: A Mast Cells-Associated Disorder?**

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**Background:** Studies have shown that mast cells and other immunocytes are increased in the colonic mucosa of adult patients with irritable bowel syndrome (IBS). Several lines of evidence support the hypothesis that a low-grade mucosal inflammatory process, albeit undetectable endoscopically or with conventional histology, may play a role in IBS pathogenesis. We recently noticed increased number of mast cells in the colonic mucosa of pediatric patients who presented with non-specific abdominal pain. The role of colonic mucosal mast cells in children with non-specific abdominal pain has not been previously explored.

**Design:** Pediatric patients presented with non-specific abdominal pain, normal colonoscopy findings and apparently normal colon biopsies histology. The control group consisted of 12 autopsy cases of patients who died because of isolated central nervous system diseases. Colonic biopsies from patient and control groups were stained with CD117. In each region of the colon, the area with the highest number of mast cells (hot spot) was selected and the number of mast cells was counted per one high power field (X400).

**Results:** The patients group consisted of 36 patients (median age 11.22 years). The control group consisted of 12 autopsies (median age 10.3 years). Clinical presentations of the patients group included abdominal pain, abnormal bowel movement, blood in stools and joint pain with the most common diagnosis being recurrent abdominal pain (70%) and the most common symptom (after pain) being chronic diarrhea. Histologic examination of both groups showed no pathologies beside increased number of mast cells in the patients group. Details of the numbers of mast cells in various parts of the

colon in the patients and controls groups along with the corresponding P value are in Table 1.

Table 1

Anatomic location	Patients Group		Controls Group		P-value
	Median	Range	Median	Range	
Cecum	22	10-39	5	2.3-8.4	<0.0001
Ascending colon	23	8-42	5.6	4.4-7.9	<0.001
Transverse colon	22	4-35	4.5	2.2-9.1	<0.001
Descending colon	20	6-37	6.1	3.8-9.3	<0.0003
Recto-sigmoid	22	6-40	8.3	4.1-9.8	<0.004

**Conclusions:** There was pancolonic increased number of mast cells in the patients group compared to the controls group. These findings are similar to those reported in the adult population with IBS. It is tempting to hypothesize that these patients may benefit from anti-histamines or other mast cells stabilizers.

**1764 Activating ALK Tyrosine Kinase Domain Mutations in Neuroblastoma**

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**Background:** Several groups have recently reported germline and somatic mutations in the anaplastic lymphoma kinase (ALK) gene in familial and sporadic cases of neuroblastoma (NB), respectively, with the mutation prevalence ranging from 6 to 11% in sporadic NB. Activating ALK mutations were located in the tyrosine kinase domain (TKD) encoded by exons 20 through 25. Codons F1174 in exons 23 and R1275 in exon 25 were targeted by 78% of the mutations in the initial reports. The ALK locus has also been previously reported to be amplified in NB with or without MYCN amplification. Both MYCN and ALK are located on the short arm of chromosome 2, at 2p24 and 2p23.2, respectively.

**Design:** We searched for mutations in exons 20 through 25 of ALK in a panel of 191 NB, none of which were known to be familial. PCR amplification of the 6 exons encoding the ALK TKD was performed in 2 multiplex PCRs. PCR products were purified and sequenced with forward primers. All positive cases were re-amplified and sequenced in both forward and reverse directions. Normal tissue from 17 ALK mutation positive patients was also tested to investigate the somatic vs. germline origin of the mutation.

**Results:** We identified ALK mutations in 24 cases (12.6%) (Table 1). The P1191H mutation has not been previously reported and was found to be a germline heterozygous mutation in this patient. The other 16 normal tissues tested were negative for mutations, supporting ALK mutations as somatically acquired in these patients. No mutations were found in exons 20, 21 or 22. MYCN copy number data were available in all cases, of which 41/191 (21.5%) were amplified. Seven of the 24 ALK mutant cases had MYCN amplification (p=n.s.).

**Conclusions:** Our results confirm the presence of ALK mutations in a significant minority of NB, including a novel germline P1191H mutation. ALK mutations show no relationship to MYCN amplification. Testing for ALK mutations will help identify patients with NB who may be candidates for trials of ALK-directed kinase inhibitors.

Table 1. ALK mutation results

ALK mutation	Number of Cases
Exon 23 F1174L (TTC>CTC)	2 (8.3%)
Exon 23 F1174C (TTC>TGC)	1 (4.1%)
Exon 23 F1174L (TTC>TTA)	8 (33.3%)
Exon 23 F1174L (TTC>TTG)	2 (8.3%)
Exon 23 P1191H (CCC>CAC)	1 (4.1%)
Exon 24 F1245V (TTC>GTC)	1 (4.1%)
Exon 24 F1245C (TTC>TGC)	1 (4.1%)
Exon 25 R1275Q (CGA>CAA)	7 (29.2%)
Exon 25 R1275L (CGA>CTA)	1 (4.1%)
<b>Total</b>	<b>24</b>

**1765 Sampling the Placental Insertion Site Improves Funisitis Sensitivity: Implications for Maternofetal Cross-Talk**

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**Background:** Acute chorioamnionitis is a sensitive sign of intra-amnionic infection. Maternal neutrophils infiltrate the placental chorionic plate and membranes. In contrast, funisitis is a specific sign of infection, mediated by fetal neutrophils. We hypothesize that if maternal cytokine signaling plays a role in stimulating funisitis, the distal placental insertion site may be the most sensitive section to detect this fetal inflammatory response.

**Design:** We performed a prospective study of placentas collected at OHSU from October 2008 to October 2009 (n=509). The proximal fetal and distal placental insertion sections of the umbilical cord were sampled for histologic examination. Only singleton placentas were included and cases were excluded if the cord was not adequately sampled, the cord was detached, short (less than 10 cm), or abnormally inserted (eg. velamentous). Sections of the cord were scored for funisitis and arteritis. Sections of the placentas were scored for signs of infection, which required at least acute chorionitis (reported negative predictive value of 97%). All slides were reviewed by a placental pathologist (tkm) and pathology resident (avs). Their diagnoses were compared by kappa statistic to test agreement between pathologists and between the fetal and placental sections of the cord.

**Results:** The frequency of intra-amnionic infection in our cohort was approximately 35% (acute chorionitis 13% [n=67], chorioamnionitis 21% [n=109], necrotizing chorioamnionitis 1% [n=5]). Funisitis was seen in 14% of our cohort. We observed excellent diagnostic agreement between pathologists (kappa 0.63, p<0.0001), but only moderate agreement between the fetal and placental sides of the cord (kappa 0.46; TABLE). Using acute chorionitis as the gold standard for infection, the sensitivity of detecting funisitis at the placental side of the cord was 97% (95% CI, 90-99) and 65% at the fetal side (52-75).

	Proximal Funisitis (n=24)	Proximal Arteritis (n=23)	Proximal Negative (n=26)*
Distal Funisitis (n=38)	16	5	17
Distal Arteritis (n=33)	6	18	9
Distal Negative (n=2)	2	0	0

**Conclusions:** Our results demonstrate that there is a significant improvement in detecting funisitis when the umbilical cord is sampled near the placental insertion site. The reason is uncertain, but we postulate that cytokine signaling from maternal neutrophils infiltrating the chorionic plate may stimulate fetal-mediated funisitis in addition to the fetal response to intra-amniotic infection.

**1766 GATA-4 and FOG-2 Expression in Pediatric Ovarian Sex Cord-Stromal Tumors (SCST): An Italian Multi-Institutional Cooperative Study**  
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**Background:** GATA-4 is a transcription factor regulating cell differentiation and proliferation: in the ovary it controls the expression of  $\alpha$ -inhibin and anti-Müllerian Hormone (MIH). FOG-2 activates or inhibits the transcriptional activity of GATA-4, counteracting the trans-activation of the MIH gene by GATA-4 in fetal ovary. A prognostic role of GATA-4 in adult ovarian Granulosa Cell Tumor has been reported. Pediatric SCST are less than 8-10% of all ovarian tumors, with the most frequent histotypes represented by Juvenile Granulosa Cell Tumors (JGCT) and Sertoli-Leydig Cell tumors (SLCT). We explored the potential pathogenetic and prognostic role of FOG-2 and GATA-4 in pediatric SCST.

**Design:** Histological slides from 15 SCST entered into TREP-study (an Italian multi-institutional study for rare tumors) were reviewed, and immunostains for GATA-4, FOG-2 and  $\alpha$ -inhibin were performed. Clinical information were retrieved from TREP files.

**Results:** Clinico-pathologic features are summarized in the table. Mean age was 112 months (range 7-224); 1 tumor was bilateral, 12 were stage I, 2 stage II, 1 was metastatic at diagnosis. Mean follow-up was 26 months.

Table 1

Histology	FOG-2	GATA-4	$\alpha$ -inhibin	Follow-up
6 JGCT	5/6	1/5	3/6	6 I CR
6 SLCT	4*/6	2/4	5/6	4 I CR, 1 II CR (after metachronous ovarian tumor), 1 DOD
FT/SST	2/3	1/1	0/3	3 I CR

\*only Sertoli component. Legend: I/ICR=first/second complete remission, DOD=died of disease. All were treated by surgery, with adjuvant chemotherapy in 3. There were 6 JGCT, 6 SLCT, 2 Fibroma/Thecoma (FT) and 1 Sclerosing Stromal Tumor (SST). SLCT behaved more aggressively: 1 SLCT (FOG/GATA negative) was metastatic at diagnosis, and the patient is in I CR; one poorly differentiated SLCT with fatal outcome and 1 metachronous bilateral SLCT were GATA-4 and/or FOG2 positive.

**Conclusions:** Compared to adult GCT, JGCT express FOG-2, but are frequently GATA-4 negative, suggesting a possible switch to the phenotype of the fetal ovary. The favourable prognosis in most pediatric SCST does not allow to draw a conclusion on the prognostic role of FOG-2 and GATA-4. Therefore, absence of GATA-4 in JGCT may contribute to their favourable prognosis. In SLCT, GATA-4 and/or FOG-2 were expressed in more aggressive tumors. There was no relationship between FOG-2/GATA-4 and  $\alpha$ -inhibin staining.

## Pulmonary

**1767 Are Cytology Blocks Adequate for EGFR Mutational Testing? An Institutional Experience**

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**Background:** EGFR mutation status has been shown to predict response to anti-EGFR tyrosine kinase inhibitors in non-small cell lung cancer (NSCLC). In patients with advanced stage NSCLC, surgical resection is not part of routine care, therefore evaluation of mutational status is increasingly requested on biopsy or fine needle aspiration (FNA) specimens, in which the available material is limited. There are limited data on the suitability of cytology cell blocks for EGFR mutation testing. In this study we report our institutional experience with cytology cell block material for EGFR mutation testing.

**Design:** We retrospectively reviewed EGFR mutation analyses performed on 135 surgical (SP) and cytology cell blocks (CB) from Oct. 2007-Sept. 2009. One hundred fifteen (115) SP and 20 CB specimens were evaluated for L858R and exon 19 in-frame deletions (analytic sensitivity ~5%). Cytology cell block specimens were evaluated for overall specimen size (total cellularity) and percent tumor. Percent tumor was scored as <5%, 6-10%, 11-25%, 26-50%, >50%. Immunohistochemistry for TTF-1 and CK7 were used to assist in assessment of tumor percentage, when available. Demographic features such as gender and smoking status were evaluated, as EGFR mutations are more frequently seen in women and non-smokers.

**Results:** Of the 115 SP and 20 CB specimens, 19 (16.5%) and 7 (35%) were positive for EGFR mutation, respectively. The mutation rates were not statistically different between the surgical and cytologic specimens (p=0.065). Of the 20 CB, half were <2mm<sup>2</sup>; of the 7 cases with a mutation, 4 (57%) were <2mm<sup>2</sup>. Limited DNA (<25ng/uL) was obtained from 70% (14/20) of CB specimens, including 71% (5/7) of those which were mutation positive; additionally, 57% (4/7) of the positive FNA specimens had extremely low DNA yields (<6.25ng/uL). 20% (4/20) of all FNA specimens had

<10% tumor, however all 7 of mutation positive cases had >10% tumor. There were no differences between the SP and CB specimens with regard to patient gender or smoking status (p=0.31 and p=0.27 respectively).

**Conclusions:** Targeted mutational testing was successfully performed in CB specimens, even when scant. These data indicate that CB specimens provide an alternative source for molecular evaluation of NSCLC, and that tumor percentage may be more important than DNA yield in determining the suitability of these specimens for testing.

**1768 Distinguishing Primary from Metastatic Squamous Cell Carcinoma of the Lung in Patients with Known Head and Neck or Gynecological Tract Carcinoma**

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**Background:** Determining if a lung squamous cell carcinoma (SCC) is a primary tumor or metastatic process in patients with concurrent or prior extrapulmonary SCC is challenging. In most cases, the prior SCC originates from the head and neck (H&N) or lower gynecological tract. Since a significant proportion of these tumors are HPV related, we used a panel of immunostains (IHC) for p16, p53, as well as *in situ* hybridization (ISH) for high risk HPV, to determine the relationship between the pulmonary and extrapulmonary tumors.

**Design:** Twenty-seven patients (14 males; mean age 58 years, range 42-77 years) with SCC of the lung as well as an extrapulmonary SCC were identified. Four extrapulmonary tumors were from the gynecologic tract (3 cervix, 1 endometrium) and 23 cases were from the H&N. All specimens were stained with p16 and p53 (for p16: diffuse staining, cytoplasmic and/or nuclear was considered positive; for p53: more than 5% of nuclei stained was considered positive). Negative cases for p16 were tested with ISH for HPV DNA. Pulmonary specimens with HPV-ISH or p16 status diverging from that of the extrapulmonary site were interpreted as primary SCC. When HPV-ISH or p16 status was positive in both sites, pulmonary SCC was considered to be metastatic. In cases with negative HPV-ISH and p16 status, divergent results for p53 were interpreted as primary SCC of the lung, while similar p53 results were considered inconclusive.

**Results:** In 9 (33%) cases (3 cervical and 6 H&N), the pulmonary and extrapulmonary tumors had positive HPV status, and the lung SCCs were considered metastases, while 6 (22%) SCCs (all H&N) had different HPV status and the lung SCCs were determined to be primary in origin. In 3 cases, (2 H&N and 1 endometrium) both the extrapulmonary and pulmonary tumors were negative for HPV but with divergent p53, suggesting primary pulmonary origin. In 9 cases (all H&N) both sites were HPV negative and showed similar p53 staining; a definitive classification of primary or metastasis could not be determined by IHC alone. Overall, we were able to determine primary vs metastasis in 67% of the cases (100% gynecological and 61% H&N).

**Conclusions:** Our study supports a panel of immunostains for p16, p53 and HPV ISH as a useful tool to distinguish between primary and metastatic pulmonary SCC. These results raise the importance of markers that will distinguish pulmonary lung SCC from a metastasis, especially in HPV negative cases.

**1769 Characteristic Morphology and Immunoprofile of Lung Adenocarcinoma with KRAS Mutations: Propensity for Solid Growth Pattern and Correlation with TTF-1 Expression**

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**Background:** KRAS mutations in lung adenocarcinoma (AD) define a distinctive clinical subgroup of patients who are resistant to not only to EGFR-targeted therapies, but also to conventional chemotherapy. Compared to EGFR-mutant tumors, KRAS mutations are strongly associated with smoking, and are predictive of poor prognosis. However, whether KRAS mutations define a morphologically and immunophenotypically distinctive subtype of AD has not been established.

**Design:** 82 resected lung AD were analyzed for KRAS and EGFR mutations, and classified histologically based on the presence or predominance (>50%) of solid vs other (bronchioloalveolar, acinar, papillary, micropapillary) patterns. All tumors were evaluated by immunohistochemistry for TTF-1 as positive (any reactivity) or negative.

**Results:** Of 82 tumors, 27 (33%) harbored KRAS mutations, 17 (21%) EGFR mutations, and 38 (46%) neither KRAS nor EGFR mutations. KRAS mutations were strongly associated with solid-predominant growth pattern: 16/25 (64%) of solid-predominant tumors were KRAS-mutant compared to 11/57 (19%) of non-solid predominant tumors (P=0.0002). Even tumors with a minor solid component had more frequent KRAS mutations (21/45, 46%) than tumors without any solid component (6/37, 16%; P=0.004). In contrast, none of solid-predominant tumors had EGFR mutations (0/25) as compared to tumors with other patterns (17/57, 30%; P=0.001). TTF-1 was expressed in 26/27 (96%) of KRAS-mutant tumors, 17/17 (100%) of EGFR-mutant tumors, and 31/38 (82%) of non-mutant tumors. Presence of either KRAS or EGFR mutations was significantly associated with TTF-1 expression (P=0.02).

Correlation of KRAS and EGFR mutations with solid pattern in lung AD.

Histologic Type	KRAS-mutant	EGFR-mutant	KRAS and EGFR non-mutant
All types combined (n=82)	27 (33%)	17 (21%)	38 (46%)
Solid predominant (n=25)	16 (64%)	0 (0)	9 (36%)
Any solid (n=45)	21 (47%)	7 (15%)	17 (38%)

**Conclusions:** We find that solid growth pattern, particularly if predominant, is a characteristic histopathologic feature of KRAS-mutant AD. This pattern has been previously shown to correlate with aggressive behavior, consistent with the poor prognosis of KRAS-mutant tumors. Correlation with TTF-1 expression has been previously established for EGFR-mutant tumors, and we extend this observation to tumors with KRAS mutations.