Conclusions: Based on these data, we hypothesize that decreased fucosylation impairs the interaction between tumor cells and their external milieu, which in turn, affects key cell functions modulating tumor progression. Decreased adhesion on HUVEC in the presence of fucosidase also evokes the rationale that defucosylation may modulate metastasis, and thus provides a promising approach to hinder tumor progression and dissemination.

1375 Effects of a Monoclonal Anti-αvβ3 Antibody on Blood Vessels – A Pharmacodynamic Study

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Background: Angiogenesis is required for wound healing and tumor growth. The integrin αvβ3 is an adhesion molecule expressed by proliferating endothelial cells and antibodies blocking this integrin inhibit angiogenesis in pre-clinical models. Focal adhesion kinase (FAK) is an intracellular signaling molecule, which forms a complex with integrins and growth factor receptors and thus integrates signals generated by both receptor types. MEDI-522 is a second generation humanized monoclonal anti-αvβ3 antibody designed as a therapeutic anti-angiogenic agent. The purpose of this correlative study was to determine the distribution of MEDI-522 in tissues and to examine potential effects on blood vessels.

Design: In a phase I dose escalation study, MEDI-522 was administered by weekly infusions to 25 adult patients with advanced solid organ malignancies. As a surrogate angiogenesis assay, a wound was created by punch biopsy of the arm skin. This wound site was re-biopsied after a 7-day interval. Sequential pre-treatment and four-week treatment biopsy pairs were available on four patients, who had received 6 or 10 mg/kg of MEDI-522. Localization of the therapeutic agent, levels of β3 integrin subunit, vascular density, proliferation and apoptosis of endothelial cells, and phosphorylation state of focal adhesion kinase (p-FAK) were determined by dual-label immunofluorescence and computer-assisted image analysis.

Results: Medi-522 was detected in the perivascular area as well as the dermal interstitium both in intact and wounded skin sites following treatment. No statistically significant difference was found between pre-treatment and treatment samples of skin for vascular density, proliferation and apoptosis of endothelial cells, and αvβ3 integrin levels. However, the immunofluorescence intensity of p-FAK was significantly lower in skin wound vessels during MEDI-522 treatment compared to the pre-treatment samples.

Conclusions: Medi-522 was detectable both in quiescent and in angiogenically active skin blood vessels as well as in the dermal interstitial space. The levels of phosphorylated FAK were reduced during Medi-522 treatment, suggesting a modulating effect on this signaling molecule. Work supported by the National Cancer Institute, UO1 CA62491 and NO2-CO-124001 (22XS082A)

1376 Transgenic Mice Overexpressing Human 8-Oxoguanine DNA Glycosylase (hOGG) in Mitochondria Develop Obesity, Hepatosteatosis and Female Infertility

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Background: Mitochondria are dynamic organelles that play critical roles in oxidative phosphorylation and energy metabolism. Mitochondrial DNA damage and dysfunction play vital roles in the development of a wide array of mitochondria-related diseases, such as obesity, diabetes, infertility, and malignant tumors in human. Here, we reported the generation of a transgenic (TG) mouse model of human mitochondrial diseases by overexpressing hOGG1, a base excision DNA repair gene, in the mitochondria of a wide variety of tissues in mice.

Design: hOGG1 TG mice were produced by pronuclear microinjection using a mammalian expression vector consisting of a mitochondrial isoform of the human OGG1 full-length cDNA under the regulation of mouse metallothionein promoter 1 (mMT-1). Transgene integration was analyzed by PCR. Gene expression was measured by real-time RT-PCR and western blot analysis. Mitochondrial DNA damages were analyzed by direct DNA sequencing and real-time quantitation of mitochondrial copy number. Total fat content was measured by whole body scan using Dual Energy X-ray Absorbtiometry.

Results: The hOGG1 TG mice express very high levels of human OGG mRNA (50 to 1000 folds as high as endogenous mouse OGG) in almost all organs analyzed with the liver being the highest expressing organ. Significantly more mitochondrial DNA mutations and less mitochondrial copies per cell were seen in hOGG1 TG mice. hOGG1 TG mice become obese from 2 months of age as manifested by increased body weight and whole body fat percentage. Diffuse steatosis develops progressively with age. The female TG mice are infertile. At about 2 years of age, increased frequencies of hematopoeitic malignancies are seen in these TG mice.

Conclusions: Defects in mitochondrial genome and function have profound adverse biological effects in mice, resulting in obesity, hepatosteatosis, female infertility and the development of hematopoeitic malignancies, presumably brought about by the disruption of energy and fatty acid metabolism and increased ROS production.

1377 Mitochondrial DNA Damages Enhance the Cell Killing Effects of Cisplatin in Human Hepatoma Cells *Via* Increased Intracellular Free Radical Production

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Background: Cancer cells are constantly under oxidative stress and susceptible to free radical-induced apoptosis. Defect in mitochondrial respiration has been shown to cause increased free radical production and, consequently, enhanced sensitivity to apoptosis-induced chemotherapeutic agent in cancer cells. Overexpression of base excision repair gene in cells will cause imbalance in base excision repair, leading to paradoxically increased DNA breakages. In this study, we explore a novel strategy of enhancing cell killing effect in human hepatoma cells by Cisplatin by overexpression of hOGG1, a base excision DNA repair gene in the mitochondria of a human hepatoma cell line.

Design: The mitochondrial isoform (2a) of hOGG1 gene was overexpressed in a human hepatoma cell line (HepG2). The expression of mMT-hOGG1 transgene was measured by RT-PCR and fluorescent immunohistochemistry. Mitochondrial DNA deletion was analyzed by long-range PCR. The amount of free radicals (hydrogen peroxide and superoxide) was measured by flow cytometric analysis. Apoptosis was measured by flow cytometric analysis. Responses to chemotherapy (Cisplatin) in these cells were determined by colony formation experiments.

Results: mMT-hOGG1 transfected hepatoma cells (H8) expressed high levels of transgene mRNA as compared to the control cells by RT-PCR and the protein product of the expressed transgene was targeted successfully to the mitochondria in H8 hepatoma cells by fluorescent immunohistochemistry. There were much enhanced mitochondrial DNA deletions and production of free radicals (hydrogen peroxide and superoxide) in the H8 cells as compared to the control hepatoma cells. H8 hepatoma cells underwent more active apoptosis and consequently, more susceptible to the cell killing effects of Cisplatin, a common chemotherapeutic agent used in the treatment of liver cancer.

Conclusions: Targeting of hOGG1 gene expression to the mitochondria in human hepatoma cells increases the sensitivity of cancer cells to the killing effects of Cisplatin through enhanced mitochondrial DNA deletions, production of free radicals, and increased intrinsic apoptosis. The violation of mitochondrial DNA integrity or disruption of its function, thus represents a novel mechanism upon which more effective chemotherapeutic strategies can be developed in the treatment of human cancer.

1378 Cell Surface Molecule R9.14 Is Expressed by Monoblastic Leukemia of FAB M5 Type as Well as Reed-Sternberg Cell Lines and Anaplastic Large Cell Lymphoma Cell Lines

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Background: Anti-R9.14 is a murine monoclonal antibody raised in the Banerjee laboratory against KMH-2 cells, a Hodgkin lymphoma-derived cell line. During testing of the specificity of the antibody, it was observed that, unlike all other antibodies raised against KMH-2 cells, anti-R9.14 labelled not only CD30+ Hodgkin and Anaplastic Large Cell lymphoma cell lines, but also labelled a CD30 negative monoblastic cell line U937. This led to the investigation of the expression of R9.14 on leukaemic blasts from patients with newly diagnosed acute myeloid leukemia (AML).

Design: Antigen expression was studied by flow cytometry and the antigen is further identified with Western-blot analysis.

Results: Of 16 AML patients studied by flow cytometry, the CD45 dim blast cells expressing R9.14 were determined to be only in patients with acute monoblastic leukemia (AMoL) of both FAB M5a and FAB M5b categories. Blast cells of other FAB types did not express the epitope. R9.14 is also expressed by monocytes but not normal CD34+ bone marrow precursors, granulocytes, lymphocytes or erythroid precursors. By Western blot analysis R9.14 is a ~92 KDa molecule. Anti-R9.14 has a pleiotropic effect on cell proliferation, depending upon the cell line and the incubation time.

Conclusions: R9.14 may be functionally significant in monoblastic leukemia, Hodgkin lymphoma and Anaplastic Large Cell Lymphoma. Molecular cloning and sequencing of the R9.14 gene will be undertaken.

Pediatrics

1379 Portal Vein Alterations in Patients with Extrahepatic Biliary Atresia

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Background: Vascular lesions of the liver are protean and may be observed in several conditions; **arterial** lesions have been observed in extrahepatic biliary atresia (EBA). Alterations of the portal **vein** or its main branches, to our knowledge, have not previously been described. Peculiar portal vein lesions found in a group of patients with EBA are here informed.

Design: This is a descriptive, retrospective study of 62 consecutive biopsies of patients with EBA who underwent porto-biliary anastomosis, in a ten years period. Vessels from the hepatic hilium were studied with H & E, Masson´s trichrome, elastic fibers, mucicarmin, alcian blue, PAS, coloidal iron, CD20, CD3, CD68, and actin stains. Celular or fibrous subendothelial proliferation, elastica damage, edema, calcification, thrombosis, and glycosaminoglycan deposits were searched; the extention of the damage was graded as mild (less than 25% of circumference), moderate (26 to 50%), or severe (over 50%). Vessels were classified according to their circumference as medium sized (less than 300 microns) and large; when possible the portal vein and/or its larger branches were evaluated. Hepatic explants (n=20) of patients without EBA were used as controls. Demographic data were obtained from clinical charts.

Results: Age averaged 3 months; 45 patients were females. In 24 cases portal vein or its larger branches were present. Vascular alterations were observed in 35 cases: arterial changes in 13, vein lesions in 10, and damage in both types of vessels in 12. The main arterial changes found were: elastica rupture (n=19), glycosaminoglycans deposits(n=19), and subendothelial celular proliferation (n=20); all changes were mild to moderate. On the other hand, glycosaminoglycans deposits (n=7), subendothelial fibrous proliferation (n=8), and elastic fibers rupture (n=9), were the principal alterations observed in the portal vein or its branches; all changes were mild.Subendothelial

cellular proliferation was composed of macrophages, CD20 and CD3 lymphocytes, and myofibroblasts. Those alterations were present since early ages and were not observed in the controls.
Conclusions: Vacular alterations were commonly observed in EBA, portal vein changes were present in at least one third of our cases, they appear to be part of the process and not an acquired condition, may explain some vascular complications of liver transplantation of patients with EBA, are in accordance with vascular and/or autoimmune theories en EBA origin; and may provide data for future insights on EBA pathogenesis.

1380 Composite Infantile Myofibromatosis: A Clinicopathologic Analysis and Comparison with Infantile Fibrosarcoma and Infantile Myofibromatosis

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Background: Infantile myofibromatosis (IM) is a mesenchymal disorder with different clinical forms including solitary, multicentric, and generalized with visceral involvement. A wide morphologic spectrum is encountered, with the extremes resembling infantile fibrosarcoma (CIFS) and infantile hemangiopericytoma.
Design: Clinical and histological features of 106 myofibroblastic lesions, including CIFS and IM, were reviewed and reverse transcriptase-polymerase chain reaction (RT-PCR) for the ETV6-NTRK3 gene fusion evaluated, when feasible, in order to define the clinicopathologic features of the so-called composite fibromatosis (COIM), its significance and relationship with CIFS and classic IM.
Results: Among the 106 myofibroblastic lesions, 56 were diagnosed as CIFS, 43 as classic IM, and 7 were classified as COIM. One case of CIFS, with a previous diagnosis of COIM, exhibited an unusual biphasic pattern with foci resembling IM, including whorls of primitive and spindle cells and perivascular and intravascular projections of myofibroblastic nodules. The 7 COIMS were highly cellular tumors displaying a diffuse growth of immature cells and only focal features typical of classic IM. Areas resembling CIFS were characterized by mitotically active spindle cells arranged in a herringbone pattern. Lymphocytic infiltration and necrosis were unusual. Immunoreactivity for smooth muscle actin was present in foci with myofibroblastic histologic features in four cases, and CD34 reactivity was present in more primitive cellular foci in 3 cases. The ETV6-NTRK3 transcript was absent in 2 COIMs and was present in 11/11 of CIFS.
Conclusions: Composite infantile myofibromatosis represents a morphologic variant of infantile myofibromatosis that can mimic infantile fibrosarcoma. Careful histologic evaluation to detect the typical features of infantile myofibromatosis is essential to avoid classification as infantile fibrosarcoma. Immunohistochemical analysis is not helpful in the distinction from infantile fibrosarcoma, but can highlight areas typical of infantile myofibromatosis. Molecular analysis for the ETV6-NTRK3 gene fusion is useful in composite myofibromatoses with a focally extensive herringbone pattern, even when areas of more typical infantile myofibromatosis are present.

1381 Overexpression of the MET Receptor Tyrosine Kinase in Tumors with TFE3 Gene Fusions

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Background: MET has been implicated in a number of cancers, especially in papillary renal cell carcinomas (PRCC) where it is activated by point mutations in some cases. In a previous microarray-based comparison of expression profiles of 137 samples of 5 translocation-associated sarcomas [Ewing sarcoma (ES), synovial sarcoma (SS), alveolar rhabdomyosarcoma (ARMS), desmoplastic small round cell tumor (DSRCT), alveolar soft part sarcoma (ASPS)], we observed that MET was significantly overexpressed in both ARMS and ASPS. The PAX3-FKHR fusion protein of ARMS is known to activate expression of the *MET* gene. We have recently also found that the ASPL-TFE3 fusion of ASPS induces MET expression and activation (phosphorylation) (M. Tsuda, M. Ladanyi, unpublished data). We therefore examined MET protein expression by immunohistochemistry (IHC) in ASPS and in the subset of pediatric/young adult renal cell carcinomas (RCC) that also contains *ASPL-TFE3* or other *TFE3* gene fusions (TFE3 RCCs).
Design: We evaluated MET expression by IHC (C-28 antibody, Santa Cruz) on tissue microarrays of pediatric sarcomas and adult and pediatric renal tumors. The IHC was scored on the basis of cytoplasmic labeling (0-12 scale, based upon percentage labeling multiplied by intensity) and membrane labeling (0-3 scale). Individual tumors with a cytoplasmic labeling score of ≥ 6 and/or a membrane labeling score of ≥ 1 were called MET IHC positive. The mean cytoplasmic labeling score (MCLS) and mean membranous labeling score (MMLS) were calculated for each tumor type.
Results:

	MET IHC RESULTS		
	Positive Cases/Total Cases	MCLS	MMLS
SARCOMAS:			
ARMS	6/18	3.8	0.5
ASPS (ASPL-TFE3+)	5/16	2.5	0.3
ERMS	1/12	1.6	0.1
SS	0/12	0.6	0.1
DSRCT	0/9	0.1	0.0
ES	0/12	0.0	0.0
RENAL TUMORS:			
TFE3 RCC	9/12	6.6	0.6
Adult PRCC	20/21	6.1	1.8
Adult Clear Cell RCC	19/20	3.6	1.1
Adult Chromophobe RCC	15/20	3.0	1.0
Wilms	0/29	0.9	0.0

Conclusions: MET overexpression by IHC is common in ASPS, TFE3 RCCs, and ARMS, consistent with activation of MET expression by the oncogenic fusion proteins in these cancers, which should therefore be evaluated for sensitivity to MET-specific kinase inhibitors. Although RCCs commonly express membranous MET, TFE3 RCCs also show strong cytoplasmic MET staining, comparable only to PRCCs. Finally, MET IHC may also have a limited diagnostic role in making the distinction between ASPS or ARMS and other sarcomas.

1382 Necrotising Enterocolitis Occurs In-Utero

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Background: Necrotising enterocolitis (NEC) is a frequent complication of prematurity and is attributed to either infection or hypoxia/ischaemia. While antenatal impaired umbilical blood flow is known to be predictive of NEC, the diagnosis is usually made in the neonate. NEC in stillbirths has not previously been reported.
Design: A retrospective analysis of perinatal autopsies was carried out over a four year period (2000-2003) within infants of birth weight ≥ 250 grams. The bowel in all cases was embedded in paraffin blocks in-toto using a serial longitudinal sectioning technique. Histological sections were made from either (i) all blocks or (ii) intermittent sampling of the blocks. Histological features evaluated comprised – Ulceration; blunting/shortening of the villi; mucosal necrosis; stricture formation; capillary dilatation; desquamation of epithelial cells; and inflammation.
Results: 70 cases were available for review. Abnormalities confirming NEC were identified in 11 cases. These comprised 10 stillbirths and one early neonatal death (at 20 hours of age). Placental examination was performed to evaluate for chronic uteroplacental insufficiency. This was identified in 7 of these cases.
Conclusions: The finding of NEC in stillbirths and early neonatal deaths conforms with the obstetrical data predictive of NEC as a complication of chronic uteroplacental insufficiency. The lack of more complicated signs, e.g. perforation, etc. most likely relates to the absence of extra uterine challenged bowel function.

1383 Pediatric Pheochromocytoma/Paraganglioma: Clinical, Pathologic and Molecular Genetic Study

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Background: The behavior of paragangliomas, whether adrenal (pheochromocytoma) or extra-adrenal, sporadic or familial, is difficult to predict. In children, this problem is compounded by a lower incidence and lack of a comprehensive clinicopathologic study. It is therefore unclear whether the proposed histopathologic criteria and scaled score for predicting malignant behavior in adult tumors can be applied to their pediatric counterparts.
Design: This study reviews clinical, genetic and pathologic features of 37 pediatric paragangliomas including 21 pheochromocytomas in 34 children. Clinical information and follow-up were obtained. Thirty-four distinct histologic features including 15 WHO criteria were assessed in all tumors by two pathologists and the WHO scaled score was determined. Direct sequencing for VHL, SDHB, SDHD and RET mutations was performed in 11 tumors for which frozen tissue was available.
Results: The patients ranged from 4 to 20 years (mean 12.9 years). Four had bilateral pheochromocytomas and two had multiple paragangliomas. Seventeen (50 %) had a syndrome associated with a genetic susceptibility, including neurofibromatosis-1 (1), Carney syndrome (1), von Hippel-Lindau (10), familial paraganglioma syndrome type3/SDHB (1), and other uncharacterized familial pheochromocytomas (4). The tumors were well-encapsulated and had fewer amphophilic cells and hyaline globules as compared to adult tumors. VHL and SDHB-related tumors tended to have a thicker capsule, desmoidal fibrosis and absence of hyaline globules, in comparison to sporadic tumors and those occurring in other genetic backgrounds. Of the 21 pheochromocytomas, 12 (57%) had histologic features suggestive of malignant potential (WHO scaled score of ≥4); however, there was only one metastasis in this group. Follow-up ranged from 4 months to 23 years (mean 5.7 years). Four patients (10%) had local recurrence and two (5%) developed metastases.
Conclusions: Pediatric tumors are well-encapsulated and have fewer amphophilic cells and hyaline globules as compared to adult tumors. VHL and SDHB-associated tumors have distinct histopathologic characteristics by tending to have a thicker capsule, desmoidal fibrosis, and absence of hyaline globules. Given the low incidence of malignancy in pediatric tumors, the proposed WHO criteria may overestimate aggressiveness in this age group.

1384 *MYCN, MYC, MDM2*, and *MDM4* Gene Copy Number Gains in Retinoblastoma

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Background: Multiple oncogenes involved in the control of cell proliferation and differentiation have been implicated in the tumorigenesis of various pediatric round blue cell tumors. *MYCN* amplification occurs in neuroblastoma and medulloblastoma; in both tumor types, this alteration confers a poor prognosis. Recent studies have identified a number of non-random regions of chromosomal gain in retinoblastoma, with 1q and 2p of particular interest as they harbor *MDM4* and *MYCN,* respectively. Proteins encoded by these genes and their homologues *MDM2* and *MYC* have been shown to interact with pRB, providing potential alternate means of RB pathway dysregulation.
Design: We retrospectively reviewed the clinical course and histologic features of retinoblastomas from 48 children treated at our institution. Fluorescence in situ hybridization (FISH) was performed on tissue microarrays containing core samples from the tumors in this cohort using locus-specific probes targeting *MYCN, MYC, MDM2*, and *MDM4* genes paired with control probes on the respective opposing chromosomal arms. Correlations were sought between clinicopathologic parameters and gene copy number status.

Results: The cohort included 29 males and 19 females, ages 0.5-7 years (mean 2.4 years); 40 patients had unilateral and 8 had bilateral disease. Gene copy number gains were documented by FISH in the following frequencies: *MDM4*-67%, *MYCN*-24%, *MDM2*-10%, *MYC*-2%. Whereas *MDM4* gain was encountered in similar proportions of low and higher stage tumors (T1-63% versus T2/3-67%), gains of *MYC* or *MYCN* were entirely limited to higher stage tumors with choroidal and/or optic nerve invasion. Combined *MYCN / MDM4* gain was detected in 20% of the samples; this molecular signature was present in 2 of 3 tumors showing scleral invasion and all 3 tumors from patients with metastatic disease, which includes 2 who expired at 8 and 50 months from presentation.
Conclusions: Gene copy number gains of *MDM4* and *MYCN*, and less frequently *MDM2* and *MYC*, are encountered in retinoblastoma, imparting alternate potential dysregulatory effects upon the RB pathway. Though *MDM4* gain may represent an earlier event in retinoblastoma oncogenesis, *MYCN* gains are limited to higher stage tumors and those showing metastatic propensity.

1385 Primary Skull Lesions in the Pediatric Population: A 25 Year Experience

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Background: Primary skull lesions are rare in the pediatric population. Previous reports suggest that dermoid and epidermoid tumors are the most common childhood skull tumors, with dermoid tumors accounting for up to 60% of these lesions. The purpose of this study was to review the clinicopathologic features of primary skull lesions identified within the pediatric population of an academic tertiary medical center over a 25 year period.
Design: A retrospective review from January 1980 to April 2005 identified 138 individuals with primary skull lesions. Twenty-one of these 138 cases occurred in individuals less than age 21 years and formed the study group.
Results: Twenty-one primary skull lesions were identified in 12 males (57%) and 9 females (43%) who had a median age at diagnosis of 10 years (range 9 weeks-20 years). Detailed clinical information was available in 15 patients. The skull lesions presented as a painless mass (n=8), a painful mass (n=3), soft tissue swelling (n=1), headaches (n=1), or as an incidental finding on imaging studies (n=2). The lesions were located in the occipital bone (n=7), frontal bone (n=5), parietal bone (n=2), and temporal bone (n=1). Intracranial extension was identified in 1 case of a cavernous hemangioma. One individual had Smith-Lemli-Opitz syndrome and one had a bicuspid aortic valve diagnosed prior to presentation. Treatment in all cases involved complete surgical excision of the lesion. Diagnoses included eosinophilic granuloma (n=7), epidermal inclusion cyst (n=5), epidermoid cyst (n=2), cavernous hemangioma (n=2), dermoid cyst (n=1), osteoblastoma (n=1), giant cell fibroblastoma (n=1), infantile myofibroma (n=1), and fibroma (n=1). Recurrence was known to have occurred in 2 individuals: 11 months post-operatively in a male diagnosed with osteoblastoma, and 18 months post-operatively in a male diagnosed with fibroma.
Conclusions: Primary skull lesions are rare in the pediatric population, comprising only 15% of all primary skull lesions identified at an academic tertiary medical center over a 25 year period. In our experience, these lesions are usually benign and most commonly present as a painless mass. Dermoid/epidermoid cysts (n=8) and eosinophilic granulomas (n=7) were the most commonly encountered lesions, together comprising over two thirds of cases. Intracranial extension is rare and with complete surgical excision of these lesions recurrence is uncommon.

1386 DNA-Dependent Protein Kinase Component (Ku86) Expression in Pediatric Osteosarcomas Prior to and Following Chemotherapy

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Background: DNA-dependent protein kinase (DNA-PK) complex (Ku86), is required for telomere protection, length maintenance, and DNA double-strand break repair. Ku86 deletion induces premature aging and apoptosis, due to its role in telomere capping, and effective and accurate DNA repair. Overexpression may lead to neoplastic transformation.
Design: Pediatric osteoblastic (n=16) and chondroblastic (n=6) osteosarcomas of long bones comprised the study population. Formalin-fixed, paraffin-embedded tissues from the primary tumor biopsies and resections following chemotherapy were available for Ku86 immunocytochemical analysis (anti-Ku86, Santa Cruz Biotechnology). Immunocytochemical expression was graded based upon the proportion of tumor cells that were immunoreactive with Ku86 antibody (trace <1%; 1+ = 1 to 25%; 2+ = 26 to 50%; 3+ = 51 to 75%; 4+ = >76%).
Results: The study population consisted of 12 females and 10 males (mean age 14.2yrs). The tumors occurred in femurs (71%) and tibias (29%). Ku86 immunoreactivity was present with all tumors. Prior to chemotherapy, the osteoblastic osteosarcoma biopsies had Ku86 expression grades of: trace=0/16, 1+ = 2/16, 2+ = 2/16; 3+ = 6/16; 4+ = 6/16, for a mean grade of 3.0. With chondroblastic osteosarcomas, Ku86 expression grades were: trace=0/6, 1+ = 2/6, 2+ = 4/6; 3+ = 0/6; 4+ = 0/6, for a mean grade of 1.7. After chemotherapy, the osteoblastic osteosarcoma resections had Ku86 expression grades of: trace = 2/16, 1+ = 3/16, 2+ = 5/16; 3+ = 6/16; 4+ = 0/16, for a mean grade of 1.9. With chondroblastic osteosarcoma resections, Ku86 expression grades were: trace=0/6, 1+ = 2/6, 2+ = 4/6; 3+ = 0/6; 4+ = 0/6, for a mean grade of 1.7. Adjacent normal bone from these cases was only trace for Ku86.
Conclusions: A DNA-dependent protein kinase complex, Ku86, is detected at a high level in osteosarcomas, in comparison with adjacent normal bone. At initial biopsy, osteoblastic osteosarcomas possess frequent tumor cells that express Ku86 (>50% cells in most tumors). Chondroblastic osteosarcomas had ≤50% of tumor cells with Ku86 detected. Following chemotherapy, Ku86 was reduced substantially with osteoblastic osteosarcomas, but not with chondroblastic osteosarcomas. Telomere protection and length maintenance associated with this DNA-PK complex member may be a therapeutic target for osteoblastic osteosacoma.

1387 Analysis of Eosinophilic Infiltration in Reflux Versus Eosinophilic Esophagitis in Pediatric Esophageal Biopsies

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Background: Eosinophilic esophagitis (EE) is a recently described disorder that often mimics gastroesophageal reflux disease (GERD) clinically. It differs from GERD in a greater magnitude of eosinophilic infiltrate and clinical refractoriness to acid suppression. The aim of our study was to examine the pattern of eosinophilic infiltration in EE and compare it with GERD in pediatric age group.
Design: We retrospectively analyzed esophageal biopsy specimens that were diagnosed as GERD (or consistent with) or EE over an 18 month period in pediatric patients. We noted the number of eosinophils in each high power field (hpf) and calculated the average eosinophils/hpf for each biopsy specimen. All fields were plotted on individual case histograms of eosinophils/hpf versus number of hpf for each biopsy.
Results: Out of a total of 356 patients who underwent biopsy, 70 (19.6%) were diagnosed as GERD (45 male, 25 female; mean age 10 years, range 12 weeks to 20 years) and 13 (3.6%) as EE (10 male, 3 female; mean age 7.5 years, range 1 to 16 years). Of the 70 GERD cases, 15 (21%) did not have intramucosal eosinophils but had other features such as papillomatosis and basal cell hyperplasia. The average number of eosinophils/hpf in GERD was 2.4. In contrast, the average number of eosinophils/hpf in EE was 14.2. In EE eosinophils were distributed diffusely throughout the biopsies with focal microabscesses. In GERD eosinophil clusters were much less frequent and focal. 3 of the 70 GERD cases (4%) had histograms, which fell into the EE pattern and were outliers from the GERD group. All 3 of these had > 25 eosinophils in 3 or more hpf and an average of 17.6 eosinophils/hpf. Sampling of the biopsy show that the number of hpf varied from 5 to 29 (mean 14.3 hpf/biopsy) in GERD cases and 13 to 26 (mean 20.7 hpf/biopsy) in EE cases.
Conclusions: EE in pediatric patients exhibits a much higher degree of eosinophilia and mean eosinophils/hpf than GERD. Diffuse involvement of the surface epithelium is the rule in EE. Although GERD may show focal clusters of eosinophils, this is focal and not widespread. Investigation of the clinical features and follow-up of these patients may clarify the nature of the 4% of outliers.

1388 Correlation of Acute Chorioamnionitis with Antepartum Risk Factors for Sepsis and Neonatal Outcome

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Background: Neonatal sepsis is a rare complication with a high morbidity and mortality. There is no single reliable marker for diagnosis and symptomatology is subtle. It is unclear which risk factors predict histologic acute chorioamnionitis (CA) and acute funisitis (AF) and how these correlate with risk of neonatal infection.
Design: Antepartum risk factors studied were 1) history of Group B Streptococcus (GBS) colonization with inadequate antibiotic prophylaxis (IAP), 2) maternal fever > 38.5°C (MF), 3) low grade maternal fever 38.0 to 38.4°C (LGMF), and 4) premature rupture of membranes (PROM) x 18 hours. The study group consisted of 111 infants with a mean gestational age of 39.4 weeks and mean birthweight of 3340 grams. Normal controls consisted of 430 infants and placentas with no history of infection, fever, PROM or positive GBS status examined over the previous six months. Placentas were examined for the presence of CA and AF. Blood cultures were obtained on all neonates.
Results: Patients with a history of PROM had CA in 15% (3/20) and 5% (1/20) had AF; in GBS-IAP there was 29% (5/17) CA and 6% (1/17) AF; in LGMF there was 46% (19/41) CA and 17% (7/41) AF; MF had 61% (20/33) CA and 33% (11/33) AF. The incidence of CA in the normal controls was 12% (52/430), not significantly different from the incidence in patients with PROM (P=.70) but significantly different from those with MF (P<.001), LGMF (P<.001) and IAP (P=.036). In addition, MF and LGMF were significantly associated with CA when compared to other risk factors such as PROM (P=.001) and IAP (P=.01). Only MF was significantly associated with AF compared to PROM (P=.017) and IAP (P=.031). Only 2 neonates had positive blood cultures; both for GBS in mothers with a negative screen for GBS. One had a LGMF and the other had PROM. Both were treated without sequelae.
Conclusions: Although the incidence of bacteremia and sepsis is low in these infants, LGMF, MF and IAP are associated with an increased incidence of intra-amniotic infection (CA and AF), while PROM is not. Thus, placental examination is essential in patients with LGMF, MF or GBS-IAP and should be used in conjunction with neonatal screening for the diagnosis of neonatal sepsis and infection.

1389 Correlation between Histopathologic Findings and Cytokine Gene Polymorphisms in Twin Placentas

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Background: Prematurity is the leading cause of infant morbidity and mortality. Since the twin birth rate has increased approximately 50% in the US in the last 20 years and mutifetal pregnancies are at increased risk for prematurity, the impact of these complications is of growing concern. Increased serum and intra-amniotic levels of interleukins (IL), including IL-1β, IL-1ra and IL-4 are associated with intra-amniotic infection and preterm labor and delivery. Variations in the IL-1ra, IL-1β, IL-4, IL-10 and tumor necrosis factor (TNF)-α genes have been associated with pregnancy outcome. The aim of this study was to evaluate the potential associations of polymorphisms in these cytokines with placental pathology.
Design: Mucosal epithelial cells were obtained from the buccal cavity of mothers and neonates and cellular DNA was analyzed by PCR for length polymorphisms in intron 2 of the IL-1ra gene and single nucleotide polymorphisms in the IL-1β (+3953), IL-4

(-590), IL-10 (-1082) and TNF-α (-308) genes. Histopathology was available for 129 twin pregnancies and placentas were examined for abnormalities in 5 major categories: Malperfusion (M) - infarcts, increased syncytial knots, decidual vasculopathy, villous ischemic change; Inflammation (I) - acute deciduitis, acute chorioamnionitis; Cord problems (C) - abnormal insertion, single artery, knot, twisted or long cords; Fibrinoid (F) - increased perivillous fibrinoid; Thrombosis (T) - fetal thrombotic vasculopathy.
Results: There were no significant associations between placental histopathology and different gene polymorphisms in the mother. In the fetus, homozygosity for the IL-1ra 2 allele versus other combinations of alleles (P=0.004) and carriage of the IL-4 T allele (P=0.04) were both associated with acute deciduitis. No significance was noted with polymorphisms in the IL-1, IL-10 or TNF-α genes.
Conclusions: Previously it has been shown that carriage of the IL-1ra 2 allele is associated with preterm delivery and preterm premature rupture of membranes. Our study shows that fetal carriage of the IL-1ra 2 and IL-4 T alleles is associated with acute inflammation in the decidua. Carriage of these alleles may render the fetus more susceptible to inflammation and infection. Although susceptibility to infection is likely multifactorial, our study may explain the connection between these alleles and preterm delivery, as well as subsequent neonatal morbidity and mortality.

1390 Placental Pathology and COX-2 Gene Polymorphisms in Twin Gestation

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Background: COX-2 receptor expression in the placenta has been associated with placental ischemia. In addition, maternal administration of COX-2 inhibitors appears to increase placental levels of vasodilators such as prostaglandin E2 and nitric oxide and decrease thromboxane B2 levels in experimental animals. This data suggests that COX-2 is an important factor in placental perfusion. However, genetic polymorphisms in the COX-2 gene in human pregnancy and their association with placental pathology have not been studied.
Design: Buccal mucosa epithelial cells were obtained from mothers and neonates and cellular DNA was analyzed by PCR for a single G>C polymorphism at position –765 in the COX-2 gene promoter. Clinical data was obtained from medical records. Placental histopathology was available for 129 gestations and placentas were examined for abnormalities in 5 major categories: Malperfusion (M) - infarcts, increased syncytial knots, decidual vasculopathy, villous ischemic change; Inflammation (I) - acute deciduitis, acute chorioamnionitis; Cord problems (C) - abnormal insertion, single artery, knot, twisted or long cords; Fibrinoid (F) - increased perivillous fibrinoid; Thrombosis (T) - fetal thrombotic vasculopathy.
Results: There was no significant association between placental histopathologic findings and polymorphisms of the COX-2 gene in the mother. However, in the fetus, carriage of the COX-2 C allele was associated with placental ischemia/malperfusion (P=0.01). Not surprisingly, ischemic lesions were also significantly associated with intrauterine growth restriction (IUGR) in the fetus (P=0.0003). No other group of pathologic lesions was associated with polymorphisms in this gene.
Conclusions: The COX-2 C allele is associated with reduced promoter activity and decreased expression of COX-2. Our study shows that carriage of this allele by the fetus, and thus the placenta, is associated with ischemic lesions in the placenta as well as IUGR. This suggests that decreased expression of COX-2 is associated with placental malperfusion which may be of sufficient severity to lead to IUGR.

1391 Correlation between Villous Mineralization, Embryonic Demise and Aneuploidy

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Background: Basement membrane mineralization of the villi is uncommon in normal pregnancies. However, previous studies have shown that aneuploid pregnancies may show an increase in villous mineralization (VM) histologically. More recent studies attribute VM a feature of fetoplacental circulatory failure.
Design: Placentas from 224 early pregnancies with known karyotypes were examined microscopically. Fourteen cases were excluded due to insufficient villi. The remaining cases included 14 euploids, 111 trisomies, 53 X chromosome monosomies, and 32 triploids. We attempted to determine whether VM might be predictive of specific aneuploidies and to evaluate possible associations between villous morphology and presence or absence of fetal parts with the presence and degree of VM.
Results: The VM was confirmed to be iron deposition by an iron stain. VM was seen in 9 euploids (64%), 46 trisomies (41.4%), 51 X chromosome monosomies (96.2%) and 24 triploids (75%). Of the trisomies, all cases of trisomy 18 (n=11), trisomy 9 (n=5) and trisomy 17 (n=3), and 13 of 17 cases of trisomy 21 (76.4%) showed VM. An absence of VM was noted in all (100%) cases of trisomy 16 (n=19), trisomy 2 (n=5), trisomy 8 (n=5) and trisomy 20 (n=3), suggesting a predisposition to VM associated with certain karyotypes. Of the 70 cases with recognizable fetal tissue, 67 (95.7%) showed VM microscopically. Heavy mineralization was observed in 25 cases (37.3%), moderate in 22 cases (32.8%) and scant in 20 cases (29.8%). VM was seen in only 27 of 51 cases of hydropic villi with cistern formations (52.9%). A decrease in the degree of VM was also noted, reflecting the poor vascularization often seen with hydropic villi.
Conclusions: These findings demonstrate an association between specific cytogenetic abnormalities and the presence of VM. However, the mineralization does not appear to be an expression of the particular karyotype but rather may reflect the stage at which growth arrest occurs with the various aneuploidies, as the presence of fetal tissue is associated with an increase in the degree of VM. Chromosomal abnormalities resulting in early embryonic growth and poor villous vascularization are less likely to show VM. Placentas with hydropic villi usually have a lesser degree of mineralization, most probably due to scant blood vessels. This supports the fact that VM is fetal in origin and maybe the effect of the breakdown of fetal red blood cells at the time of embryonic demise.

1392 Expression of Immunohistochemical Markers in Prenatal Female and Male Gonads

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Background: Oncogenes and tumour suppressor genes that control cell proliferation and apoptosis may play a significant role in embryogenesis. Studies have examined many genes within fetal gonadal tissue, however few have undertaken a systematic examination of adult tumour marker expression. Characterization of these markers in fetal tissue will provide additional information about human gonadal development, as well as contribute to knowledge of neoplastic processes in these organs.
Design: Human fetal gonads were obtained from surgical and autopsy cases at our institution. Testes were from male fetuses of 14-40 weeks gestation (n=16) and ovaries were from female fetuses of 13-38 weeks gestation (n=15). A panel of immunohistochemical stains, including Ca-125, P16, E-cadherin, Inhibin, Calretinin, WT-1, PLAP and TTF-1 was applied to each specimen.
Results: P16, Ca-125 and TTF-1 demonstrated no expression in fetal testes or ovaries. E-cadherin was expressed by germ cells in 3/16 (18%) of fetal testes and in 14/15 (93%) of fetal ovaries. PLAP was expressed by germ cells in 9/16 (56%) of fetal testes and in 10/15 (66 %) of fetal ovaries. Germ cells and/or Sertoli cells showed nuclear staining for WT-1 in 12/16 (68%) of fetal testes. In contrast, germ cells demonstrated no WT-1 expression in fetal ovaries but the surface epithelium and stroma were positive in 12/15 (80%) of cases. Calretinin was positive in Leydig cells in fetal testes with rare expression in germ cells and/or Sertoli cells. In fetal ovaries the germ cells were positive for calretinin in 7/15 (47%) of cases. Finally, fetal ovaries showed rare expression of inhibin in theca-granulosa cells in 1/15 cases (7%), whereas germ cells and/or Sertoli cells were positive in 14/16 (87%) of fetal testes.

Table 1

MALE GONAD	PLAP	P16	E-Cadherin	Ca-125	WT-1	Inhibin	Calretinin
Germ cell/Sertoli cell	9/16	0	3/16	0	12/16	14/16	3/16
Leydig cell	0	0	0	0	0	7/16	16/16
Surface epithelial cell	0	0	0	0	0	0	0

Table 2

FEMALE GONAD	PLAP	P16	E-cadherin	Ca-125	WT-1	Inhibin	Calretinin
Germ cell	10/15	0	14/15	0	0	0	7/15
Leydig cell	0	0	0	0	0	0	0
Theca-granulosa cell	0	0	0	0	0	1/15	15/15
Surface epithelial cell	0	0	0	0	12/15	0	11/15

Conclusions: Expression of these markers is different in male versus female fetal gonads. Our observations in fetal gonads also differ from published observations in adult gonads. These contrasting patterns of expression may be related to the development and differentiation of germ cells and stromal cells.

1393 Expression of Erythropoietin and Its Receptor in Neuroblastomas

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Background: Anaemia is a potential contributor to the functional impairment that often occurs during and after cancer chemotherapy. Children with high-risk neuroblastomas treated with high-dose chemotherapy and hematopoietic stem cell support could potentially benefit from a treatment with recombinant human erythropoietin (Epo). Epo is a specific stimulator of erythropoiesis, acting via its specific receptor (EpoR), and it has been shown that EpoR is not only present in various nonhematopoietic tissues, but also in malignant adult tumors. The aim of this study was to evaluate the expression of Epo and EpoR in neuroblastomas (primary tumors and metastases) and in normal tissues.
Design: The study population consisted of 108 patients with a median age of 3 years (range, newborn to 13 years). In total, we constructed 4 blocks of tissue microarrays containing 786 (473 tissues to primary tumours, 149 to metastases (130 lymph nodes, 16 hepatic, 3 cutaneous metastases), and 164 control normal tissues (125 adrenal glands and 39 sympathetic ganglias). Immunohistochemical staining was performed using antibodies against Epo and EpoR. Among the 108 patients, 17 had a stage I neuroblatoma, 6 a stage II, 26 a stage III, 53 a stage IV and 6 a stage IVS. 36 patients died of disease and 33 developed local or metastatic recurrence. Immunostaining intensity of Epo and EpoR was evaluated by a semi-score based on the product of percentage of positive cells (0 no labelling, 1 mild labelling: <50% of positive tumoral cells, 2 moderate labelling: 50 to 80% of these cells, 3 intense labelling: > 80% these cells).
Results: EpoR and Epo were significantly differentially expressed between the tumours and the control samples (p<0.001). The expression of Epo was low in tumours (Average intensity: 0.48) and exceptional in the control group (average: 0.18), while EpoR was present with moderated intensity in neuroblastoma (average: 1.17) and low in control samples (average: 0.55). The expression of both Epo and Epo did not significantly differ between the primary tumours and the metastases. There was no significant correlation between the expression of Epo and EpoR and tumour stage.
Conclusions: Epo-R is highly expressed in neuroblastomas. It remains to define whether Epo/Epo-R has autocrine and paracrine functions in neuroblastomas, and whether exposure to Epo may stimulate tumor growth. This is currently under study.

1394 Comparative Placental Studies of Human Immunodeficiency Virus (HIV)-Positive and HIV-Negative Patients

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Background: Although the placenta is presumed to play an important role in protection against HIV transmission, the effects of HIV on the placenta are poorly understood. Studies have shown associations between maternal HIV status, adverse pregnancy outcomes, and chorioamnionitis in resource-challenged countries. However, placental

studies of HIV-positive patients in the United States are sparse. We examined a cohort of HIV-positive and HIV-negative placentas to understand the spectrum of placental inflammatory and non-inflammatory lesions.
Design: Placentas from HIV-positive patients were collected from the archives of a large inner city hospital from 1989-2004. Data were gathered on 84 HIV-positive placentas, including mother's infection status, pregnancy outcome, histological diagnoses, and neonatal outcome. 33 HIV-negative placentas submitted to pathology due to obstetrical or neonatal complications were matched by delivery dates for comparison.
Results: Patients ranged from 16-51 years old, and the majority of placentas were third trimester. The most frequent diagnosis in the HIV-positive group was chorioamnionitis (24/84 cases); seven cases were severe stage 2-3 chorioamnionitis. The next most common diagnoses were meconium deposition (12/84) and infarction (10/84). 10 HIV-positive placentas were normal histologically, while most of the HIV-negative matched placentas were normal (20/33 cases). The most frequent pathologic diagnosis of the HIV-negative placentas was meconium deposition (18/33), followed by nonspecific inflammation of the placenta (4/33). HIV-positive placentas were more likely than HIV-negative placentas to show general placental inflammation, such as chorioamnionitis, villitis, or deciduitis.
Conclusions: Our results indicate that HIV-infected placentas show a higher prevalence of chorioamnionitis and general placental inflammation than the HIV-negative placentas. These findings reciprocate similar studies in resource-challenged countries.

1395 A Role for Complement in Antiphospholipid Antibody-Associated Placental Pathology

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Background: The antiphospholipid syndrome (APS) is characterized by a triad of clinical manifestations including vascular thrombosis, elevated titers of antiphospholipid antibodies (APA), and poor obstertic outcomes such as early pregnancy loss and intrauterine growth restriction. We have previously shown a greater deposition of C4d complement protein, a marker of classical pathway activation, in the placentas of patients with APS compared to normal controls, supporting the theory that increased placental activation of complement in APS plays a role in the etiologic mechanism of this disease. We examine the placentas of patients with APS for evidence of alternative and common pathway activation, to further study the etiology of this disease.
Design: Immunohistochemistry for complement split products C3b (alternative and common pathways) and C5a-9 (common pathway), was performed on paraffin tissue sections of placentas from 48 patients with APA and from 30 normal controls. Intensity of antibody expression in villous trophoblasts (VT) is reported as none to minimal (0), moderate(1) or extensive (2); quantity of staining in the VT is reported as a percentage and the H-SCORE is calculated. Controls and cases were compared using a t-test. Intensity of expression in the extravillous trophoblasts (EVT) was uniformly strong, therefore only quantity of staining in the EVT is reported as minimal to none (0), moderate (1) or extensive (2).Controls and cases were compared using chi square.
Results: Immunoreactivity to C3b in the VT was significantly stronger in the APA cases than in normal controls (mean 59.3 vs.36.3, P=0.004). Immunoreactivity to C5a-9 in the VT was significantly stronger in the normal controls than APA cases (mean 64.5 vs.113.8, P=0.001). A significant difference was not present between cases and controls in C3b or C5a-9 staining of EVT (χ^2=2.92, P=0.232 and χ^2=2.56, P=0.110, respectively).
Conclusions: We demonstrate a significantly greater immunoreactivity to C3b protein in APA cases compared to controls; suggesting the alternative pathway, as well the classical pathway, contributes to the poor reproductive outcome associated with APS. We also report significantly increased C5a-9 deposition in normal placentas compared to APA cases. Given the allogeneic nature of fetal tissue, we expect some physiologic complement deposition, however, the significance of these results are unclear and warrant further study.

1396 Glypican 3 Protein Expression in Wilms Tumors

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Background: Glypican 3 (GPC3) is a heparan sulfate proteoglycan that can bind to growth factors promoting or suppressing cell proliferation. Recent molecular studies have shown that GPC3 mRNA is elevated in Wilms tumors (WT) suggesting its potential role in WT development. In this study for the first time we examined GPC3 protein expression in WT by immunohistochemistry.
Design: 21 cases of primary WT with corresponding normal kidney tissues and 11 cases of metastatic WT were arrayed on a single tissue microarray block. A minimum of 3 cores were acquired from each WT. Paraffin sections were subjected to immunohistochemistry with GPC3 (BioMosaics). Ki67 immunostaining was used for comparison. Cytoplasmic and membranous GPC3 and nuclear Ki67 immunoreactivities were analyzed on automated image analysis system (ACIS, Clarient). GPC3 intensity measurements were then translated into the 4-tier system as negative, weak, moderate and strongly positive staining. Ki67 nuclear indices were calculated automatically as percentages of positive nuclei.
Results: Normal kidney tissues from corresponding nephrectomy specimens were negative for GPC3 stain in 100% cases. The GPC3 immunostaining in primary WT was strongly positive in 3/21 (14%), moderately in 5/21 (24%), weakly in 5/21 (24%) and negative in 8/21 (38%) of cases. All metastatic tumors were positive with strong staining in 3/11 (27%), moderate in 5/11 (45%) and weak in 3/11 (27%) of cases. GPC3 staining was positive in blastemal and epithelial components, but negative in stromal component of WT. The GPC3 expression was 2-fold higher in metastatic tumors than primary tumors in patients with late metastasis. Moreover, 6/8 (75%) of patients with WT progression (developing metastasis and relapses) had GPC3 positive primary tumors as opposed to 6/13 (46%) of patients without signs of progression. All three patients from our cohort who died of disease had weak to moderate GPC3 protein expression in primary tumors. We found no significant correlation between GPC3 expression and proliferation rates (r=0.24).
Conclusions: In our study GPC3 protein was detected in 52% primary WT and 100% metastatic WT. We found that GPC3 expression levels increase with tumor progression and could indicate further dedifferentiation of WT. Our data suggests potential diagnostic and prognostic value of GPC3 marker although studies in larger cohorts are needed.

1397 Incidence of Vestigial Remnants in Umbilical Cords

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Background: Vestigial remnants in the umbilical cord are common findings in the microscopic examination of the placenta. Allantoic duct remnants may appear as an actual duct lined by flat or cuboidal epithelium. Occasionally, transitional epithelium may be seen. More subtle features include small epithelial foci without true lumen. Omphalomesenteric duct remnants are lined by cuboidal or columnar epithelium and may contain goblet cells. Remnants of the allantoic duct are seen more commonly than those of the omphalomesenteric duct. Previous studies have indicated that vestigial remnants are seen in anywhere from 5.9% to 23% of umbilical cords. Our data indicates that vestigial remnants are seen in up to 49% of umbilical cords.
Design: 947 umbilical cords were examined from placenta cases at Albany Medical Center (543 from 2005, 134 from 2004, 270 from 2003). The cases were pulled sequentially from each year. Each slide showed two random profiles of the umbilical cord. Umbilical cords from twins or triplets were counted individually. Each profile was reviewed for the presence of vestigial remnants. Results were scored according to whether 0,1 or 2 profiles from each cord showed a vestigial remnant and whether that remnant was an allantoic duct or omphalomesenteric duct.
Results: 483/947 (51%) of umbilical cords did not have a vestigial remnant. 464/947 (49%) of cords did contain a vestigial remnant. 438/464 (94%) of the remnants were allantoic ducts and 26/464 (6%) were omphalomesenteric ducts. In 356/464 (77%) of cords with remnants, these remnants were seen in only one of the two profiles. Both profiles showed remnants in 108/464 (23%).
Conclusions: Vestigial remnants in umbilical cords are seen more commonly than expected. 49% of the cords reviewed in this study contained remnants. Allantoic ducts were seen with a higher frequency than omphalomesenteric ducts (94% as compared to 6% in cords with remnants). Most cords had only 1 profile that contained a remnant. Previous studies have indicated a lower frequency of remnants identified in the umbilical cord. Examination of more than one profile of the umbilical cord in addition to careful evaluation of more subtle remnants such as those without actual ducts, has shown that vestigial remnants are actually present in a much higher frequency.

1398 Pituitary Adenomas in Pediatric Patients

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Background: Pituitary adenomas are relatively rare occurrences in the pediatric population, and there are few studies documenting the profile of these tumors in this age group.
Design: A retrospective clinicopathologic review of 20 pediatric patients (less than 20 years of age) with clinically presumed pituitary adenomas resected during a 24.5 year period of time (1981-2005).
Results: The 20 study patients included 11 females and 9 males. The average age at the onset of symptoms was 14 years (range 5-18 years). Only 4 patients had onset of symptoms before the age of 12 years. The majority of patients presented with headaches (N=12), visual disturbances (N=12) or, in females, menstrual dysfunction (N=7/11). All 20 patients underwent trans-sphenoidal resections. Tumor size based on radiographic data was known for 19 of 20 patients. Twelve adenomas were greater than 1 centimeter (cm) in greatest dimension; 7 were less than 1 cm. On follow-up, 3 patients had recurrent adenomas; time to recurrence was 5 months, 17 months and 70 months. Nineteen adenomas were confirmed histologically. Fourteen of these 19 adenomas were acidophilic, 4 were chromophobic and 1 was basophilic. Three contained calcifications. Fifteen had a low mitotic count (0-1 mitotic figures (MF)/ 10 high-powered fields (HPF)) and 4 had increased mitotic activity (4-5 MF/ 10 HPF). Only one had focal necrosis. One had microcystic degeneration. Immunostaining for anterior pituitary hormones was performed on the 19 adenomas. One failed to stain for any hormones. Eight stained solely for prolactin (PRL), 4 for adrenocorticotropic hormone (ACTH), and 3 for growth hormone (GH). Two stained for GH and PRL, while 1 stained for ACTH and PRL.
Conclusions: The majority of pediatric pituitary adenomas become symptomatic after the onset of puberty, and they most often present with recurrent and frequent headaches, changes in visual acuity, and, in females, menstrual dysfunction. Almost all (18/19) were secretory, with prolactinomas being the most common type. Morphologically, acidophilic adenomas predominated and the majority had low mitotic rates.

1399 Differential Expression of Epidermal Growth Factor Receptor and erbB2 in Alveolar and Embryonal Rhabdomyosarcoma

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Background: Epidermal growth factor receptor (EGFR) and erbB2 are members of the EGFR family of receptor tyrosine kinases that play an important role in tumor cell biology and constitute promising molecular targets of therapy. Expression of EGFR and erbB2 has been observed in some rhabdomyosarcoma cell lines, but their expression has not been analyzed systematically in rhabdomyosarcoma tumors. In this study, we

assessed EGFR and erbB2 expression in rhabdomyosarcoma tumors and correlated our findings with histologic subtype.
Design: Sections from two tissue microarray blocks containing 66 rhabdomyosarcoma tumors (34 embryonal rhabdomyosarcoma (ERMS), 32 alveolar rhabdomyosarcoma (ARMS)) were surveyed by immunohistochemistry using antibodies specific for EGFR (Zymed; 1:10), and erbB2 (Dako, 1:50). EGFR and erbB2 immunostains were assessed for intensity (I) (0: no immunostaining; 1: weak; 2: moderate; 3: strong) and extent (E) (percent of 1000 neoplastic cells exhibiting membranous or membranous and cytoplasmic immunostaining). Expression of EGFR or erbB2 was considered positive if I x E >20. Correlations were assessed using the Chi-square test.
Results: Patients were 38 males and 28 females with a median age of 5.6 years (range 8 months - 19 years). Expression of EGFR was identified in 31/66 (47%) cases and correlated with ERMS subtype (26/34, 76%, vs. 5/32, 16%, $p<0.0001$). Expression of erbB2 was identified in 22/66 (33%) cases and tended to be more common in the ARMS subtype (13/32, 41%, vs. 9/34, 26%, $p=0.22$). Coexpression of EGFR and erbB2 was identified in 7 tumors, most of which (6/7) were ERMS. ARMS tumors were significantly more likely to lack expression of both EGFR and erbB2, compared to ERMS (16/32, 50%, vs. 5/34, 15%, $p<0.002$).

	ERMS	ARMS
EGFR-positive	26	5
EGFR-negative	8	27

	ERMS	ARMS
erbB2-positive	9	13
erbB2-negative	25	19

Conclusions: Expression of EGFR and/or erbB2 can be detected in a sizeable subset of rhabdomyosarcoma tumors. Notably, expression of EGFR appears to correlate strongly with ERMS, which also seems more likely to coexpress EGFR and erbB2.

Pulmonary

1400 Detection of the JC Virus Genome in Lung Cancers; Possible Role of the T-Antigen in Lung Oncogenesis

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Background: JCV virus is human polyomavirus. Subclinical infection with JCV occurs in 85-90% of the population worldwide. Primary infection in early childhood. Latency in the kidney and CNS. Reactivation when the immune system is impaired. JCV genome has early sequence that contains large T – and small t-antigen, viral late genes that contain viral capsid proteins (VP1, VP2 and VP3) and the regulatory region.
Design: We investigated the presence of its genome in 62 tumors, along with 23 samples of normal lung tissue, targeting the *T-antigen*, *VP* and *Agnoprotein* by nested PCR and Southern blotting followed by direct DNA sequencing. Copy number for the *T-antigen* was calculated by real-time PCR. Immunohistochemistry was performed to assess links between p53 and β-catenin in lung cancers and the presence of viral sequences.
Results: The *T-antigen* was detected in 25 of 62 lung cancers (40.3%) but only 4 of 23 normal lung samples (17.4%) (p=0.048), *VP* in 8 (12.9%) and 4 (17.4%) and *Agnoprotein* in 16 (25.8%) and 5 (21.7%). In total, the JCV genome was present in 33 of the lung cancers (53.2%) and 10 (43.5%) of the normal samples. Furthermore, the *T-antigen* was detected in 3 of the 4 cases with lymph node metastasis (75.0%) (p=0.042) and was more frequent in adenocarcinomas than in squamous cell carcinomas (p= 0.038). Viral DNA load was 37,743.1± 99,348.0 (mean ± SD) copies/μg DNA in cancers and 89.9 ± 68.6 copies/μg DNA in normal lung tissues. Immunohistochemistry showed significant correlations between *T-antigen* and p53 (p=0.022) and also nuclear detection of β-catenin (p=0.021).
Conclusions: It is concluded that the JCV genome might be integrated in cancer cells in approximately half of all Japanese lung cancer cases, and that the *T-antigen* may play a role in oncogenesis of lung cancers through inactivation of p53 and dysregulation of the Wnt signaling pathway.

1401 Microsatellite Instability Status in Giant Cell Lung Carcinoma

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Background: Giant cell carcinoma of the lung (GCC), an unusual morphologic variant of non-small cell carcinoma (NSCLC), is an aggressive epithelial neoplasm associated with poor survival. Recent published reports suggest an association between poor outcome in NSCLC and specific genetic alterations, such as (1) high levels of microsatellite instability reflected by loss of mismatch repair genes hMLH1 and hMSH2, or (2) estrogen receptor (ER) expression. However, in GCC their significance remains unclear. Our objective was to evaluate the clinicopathologic characteristics of GCC, to investigate the inactivation of the mismatch repair genes, and determine the expression of ER, PR and βHCG in GCC.
Design: We evaluated 14 patients with GCC treated with surgery at the Brigham and Women's Hospital between 1991 and 2005. We excluded one patient with preoperative neoadjuvant chemoradiation therapy. Clinical and pathologic features were evaluated in all cases. Pathologic stage was determined according to American Joint Committee on Cancer criteria (TNM stage). Immunohistochemical studies for expression of CK7, CK20, TTF-1 protein, βHCG, ER, PR, were performed in all cases. Expression of the mismatch repair (MMR) proteins hMLH1 and hMSH2 was evaluated by immunohistochemistry.
Results: The patients were 10 men and 4 women with a median age at diagnosis of 59 years (range 39 to 87). The mean tumor size was 4.35 cm (95 percent confidence interval, 2.7-6.0 cm). The TNM stage was I for 3 patients (21.4 %), II for 5 patients (35.7%), III for 3 (21.4%) and IV for 3 patients (21.4 %). The tumor cells were diffusely positive for CK7 in all cases and for TTF-1 in 50% of cases. βHCG was positive in one case (7%) and ER in one case (7%). The tumor cells did not express PR. CK20 was focally positive in 3 cases (21%). The expression of both hMLH1 and hMSH2 was present in all cases evaluated, indicating microsatellite-stable tumors.
Conclusions: Giant cell carcinomas of the lung are unusual, large, and aggressive tumors commonly associated with lymph node and distant metastases. The present study shows that MMR deficiency and thus high levels of microsatellite instability are not involved in the pathogenesis of giant cell lung cancer. Furthermore, tumor cells do not express ER, a reported marker of poor survival present in other lung cancers. Continued studies are needed to further define factors associated with poor survival in patients with giant cell lung carcinoma.

1402 E26 Transformation Specific (ETS-1) Does Not Correlate with Prognosis in Non-Small Cell Lung Cancers (NSCLC)

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Background: ETS-1 is expressed strongly in vascular endothelial cells and adjacent interstitial cells during angiogenesis, after which it is down-regulated. ETS-1 inhibition suppresses angiogenesis. ETS-1 has been identified in NSCLC and has been associated with tumor spread and prognosis in gastric and ovarian cancers. We evaluate tumor cell and blood vessel expression of ETS-1 in NSCLC.
Design: Tissue microarrays of 340 NSCLC with 5 year or greater follow-up were immunostained with ETS-1 (1:50, Novus Biologicals, Littleton, CO) using standard immunostaining techniques. Percentage of NSCLC cell nuclei and blood vessels expressing ETS-1 were scored on a scale of 0-3 (0=no staining; 1=<33% positive; 2=33-66% positive; 3=>66% positive). %). Intensity of nuclear staining was graded as negative, weak, moderate, or strong. Results were correlated with patient survival using Kaplan-Meier analysis.
Results: Endothelial cell nuclear positivity was observed within tumor blood vessels in 23/147 (16%); 9/147 (6%) showed tumor cell nuclear positivity; and 7/147 (5%) showed both. Staining intensity was uniformly weak, and present in fewer than 33% of the cell population. No association between endothelial cell or tumor cell staining and age, sex, smoking history, tumor stage, or survival was seen. Vessel staining tended to occur less often in predominantly bronchioloalveolar pattern adenocarcinomas (BA) compared to other NSCLC (p=0.2).
Conclusions: The majority of NSCLC in our series showed no tumor or blood vessel endothelial cell staining with ETS-1, and in the 16% of cases showing staining, ETS-1 positivity did not correlate with prognosis. The presence of ETS-1 expression suggests the potential for antibody-based treatment for ETS-1 positive NSCLC.

1403 Protein Gene Product (PGP) 9.5 Immunostaining Is Inversely Proportional to Tumor Grade in Squamous Cell Lung Carcinomas

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Background: PGP 9.5 is a ubiquitin hydrolase that is typically found in neuronal tissue, and the increased deubiquitination of cyclins by PGP 9.5 may allow for uncontrolled tumor cell growth. PGP 9.5 has been identified in non-small cell lung carcinomas (NSCLC), and one study has shown PGP 9.5 expressed more in high stage (stages 3 & 4) NSCLC than low stage (stages 1 & 2) NSCLC. We evaluated the expression of PGP 9.5 in NSCLC.
Design: Tissue microarrays of 340 NSCLC with 5 year or greater follow-up were immunostained with PGP 9.5 (1:40, Research Diagnostics, Concord, MA) using standard immunostaining techniques. Percentage of NSCLC cells expressing PGP 9.5 were scored on a scale of 0-3 (0=no nuclear staining; 1=<33% positive; 2=33-66% positive; 3=>66% positive). Intensity of nuclear staining was graded as negative, weak, moderate, or strong. Results were correlated with patient survival using Kaplan-Meier analysis.
Results: >10 % PGP 9.5 expression was noted in 5/88 (6%) squamous cell carcinomas and 26/183 (17%) adenocarcinomas. PGP 9.5 staining intensity (p=0.025) and percent staining (p=0.026) were found to be inversely related to tumor grade in squamous cell carcinomas. This relationship was not observed in other cell types; however, PGP 9.5 staining intensity was greater in squamous cell carcinomas than in adenocarcinomas (p=0.04). Neither staining intensity nor percentage of tumor cells staining with PGP 9.5 showed any significant relationship to survival.
Conclusions: PGP 9.5 is not specific for neuroendocrine neoplasms. The inverse relationship between PGP 9.5 staining and tumor grade in squamous cell carcinomas differs from the results of a prior study showing increased PGP 9.5 staining in higher-grade NSCLC. The staining pattern suggests the potential for antibody-based treatment focusing on early stage squamous cell NSCLC.

1404 Cox-2 and Angiogenic Factors in Neuroendocrine Lung Tumors

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Background: Cyclooxigenase-2 (Cox-2), a known mediator of inflammation, has been associated with angiogenesis and progression in several neoplasms. The relationship between Cox-2 and angiogenic factors, such as VEGF (vascular endothelial growth factor) and its receptor VEGFR2/Flk-1 in neuroendocrine lung tumors (NELT) is not well known.