# Pediatrics

### 1400 Conjunctival Changes in Children with Kawasaki Disease: Cytopathologic Characterization

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**Background:** Kawasaki disease (KD) is a potentially fatal acute multisystem vasculitic disorder of unknown etiology. It is the leading cause of acquired heart disease in children if not detected and treated promptly. To date, a diagnostic test for the disease does not exist and the diagnosis depends on the constellation of clinical criteria, which include red eyes in addition to others. The studied pathological changes consist of coronary arteritis primarily documented at autopsy. We examined the cytopathological changes in the conjunctiva of these patients, which to our knowledge has not been done before. In order to describe these changes, a case-control prospective study was carried out.

**Design:** Bilateral conjunctival swabs were obtained from 3 groups of patients: patients with active KD (11), age-matched controls (7), and patients with inactive KD (9). The swabs were immersed in Thin Prep solution (Cytec) and smears stained by Papanicolaou stain were examined blindly by 2 cytopathologists. The cell count differential of cells was performed and recorded quantitatively using scores from 0-6 as follows: 0 (no cells), 1 (1-4 %), 2 (5-15%), 3 (16-25%), 4 (26-50%), 5 (51-75%) and 6 ( >75%). The cells observed included; non-secretory conjunctival cells, squamous cells, goblet cells, lymphocytes, neutrophils, monocytes, eosinophils, and plasma cells.

**Results:** Only neutrophils count showed a significant difference among the 3 groups. The average scores for the active KD, control group, and patients with inactive KD were 3.5, 1.6 and 1.3 respectively. One case from the active KD group showed a score of 0, believed to be an outlier for probable technical reasons and was eliminated from the analysis. Using the Pearson Chi Square test, the difference between the active KD and the inactive group was statistically significant for both eyes (right P=0.049, left P=0.004). Samples from active KD patients were more cellular. Neutrophils surrounding conjunctival epithelial cells "neutrophillic rossetting" were seen in some cases of the active disease group but not in the other groups.

**Conclusions:** "Neutrophilic conjunctivitis" is characteristic in patients of active KD. This simple minimally invasive test may be of value in the initial evaluation and subsequent follow up of such patients. Whether this phenomenon represents a primary or secondary pathologic change is uncertain, but deserves attention and further investigation.

### 1401 Xp11-Translocation Carcinomas of the Kidney as Chemotherapy-Induced Secondary Malignancies

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**Background:** Children who survive cancer are at increased risk for developing another malignancy, most commonly acute leukemias, soft tissue sarcomas, and brain tumors. Renal cell carcinoma (RCC) occurring as a second or secondary malignancy is uncommon. Xp11 translocation RCC, most commonly bearing *ASPL-TFE3* or *PRCC-TFE3* gene fusions, are a recently recognized group of neoplasms for which risk factors have not yet been identified.

**Design:** We describe five Xp11 translocation RCC that arose in young patients previously treated with chemotherapy.

**Results:** Patient 1 received chronic low dose cyclophosphamide and prednisone for systemic lupus erythematosis before he developed an *ASPL-TFE3* RCC in the right kidney as previously reported (Am J Pathol 2001;159: 179-92). Five years later, after continued chemotherapy, the patient developed a *PRCC-TFE3* RCC in the contralateral kidney. Therefore, this patient developed two clonally unrelated neoplasms with two distinct, molecularly-confirmed fusions involving *TFE3*, a previously unreported occurrence. Patient 2 developed a RCC reported to have a t(X;17)(p11.2;q25) five years after receiving cytarabine, thioguanine, etoposide, and daunorubicin for acute promyelocytic leukemia (J Ped Hem Onc 2001;23: 609-11). Review of the sections demonstrated the expected classic morphologic features of an *ASPL-TFE3* RCC, and neoplastic cells demonstrated nuclear labeling for TFE3 protein by immunohistochemistry (IHC), confirming the diagnosis. Patient 3 had a classic congenital mesoblastic nephroma treated by nephrectomy at the age of 1 month. Nine years later, he developed a contralateral radiographic renal lesion that was biopsy-negative but nonetheless treated with Vincristine, Actinomycin-D, and Adriamycin. Six months later, he was found to have an Xp11-translocation RCC in that kidney as confirmed by morphology and TFE3 IHC. Review of our files revealed one additional *ASPL-TFE3* RCC that arose in a patient treated 5 years previously for acute leukemia with VP-16, daunomycin, 6-thiogaunine, and cytosine arabinoside. Overall, 4 of 24 (17%) molecularly-confirmed Xp11-translocation RCC in our files have arisen in children treated with chemotherapy.

**Conclusions:** Cytotoxic chemotherapy may predispose to the development of Xp11 translocation carcinomas.

### 1402 Expression and Localization of ABCA3 Transporter in Neonatal Lung Disorders

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**Background:** Mutations of the gene for ABCA3, (member of ATP-binding cassette transporter family) have been recently identified in infants with fatal surfactant deficiency [NEJM,350:1296,2004]. Patients with this disorder lack mature lamellar bodies in alveolar type 2 cells (AT2C) [AJRCCM,161:608,2000], but the cellular defect and molecular mechanisms leading to surfactant deficiency are unknown.

**Design:** To investigate the expression and localization of ABCA3 in AT2C, immunohistochemical methods using specific antibodies against ABCA3 [FEBSLett,508:221,2001], surfactant protein B (SPB) and dendritic cell-lysosomal associated protein (DC-LAMP) were applied on samples of lung from age matched control infants (n=4), infants with ABCA3 mutations (n=3) and SPB deficiency (n=3). Immunoperoxidase (IP) method was used on routine paraffin sections after antigen retrieval, and double immunoflorescence (IF) labelling on frozen sections for confocal microscopy (CM).

**Results:** In lungs of controls, IP staining showed all three epitopes strongly expressed in the cytoplasm of AT2C. By CM, ABCA3 was co-localized with SPB and DC-LAMP, markers of lamellar bodies. In patients with ABCA3 mutations, IP staining showed that ABCA3 was expressed in AT2C but appeared weaker compared to controls or patients with SPB deficiency. By CM, ABCA3 was predominantly localized to the plasma membrane of AT2C and only occasionally co-localized with sparse SPB immunoreactive foci. In SPB deficiency, there was strong expression for ABCA3, whereas immunostaining for SPB, as expected was negative.

**Conclusions:** ABCA3 lipid transporter is expressed in AT2C in normal neonatal lung and is co-localized with SPB and DC-LAMP in cell organelles involved in surfactant synthesis. In ABCA3 gene mutations, while this transporter protein is expressed, its localization is likely miss-directed, leading to the defect in surfactant synthesis. Co-ordinate expression of ABCA3 and SPB is essential for the formation of mature lamellar bodies in AT2C and normal surfactant production.

### 1403 Translocations Involving the EWS Gene Locus in Ewing Sarcoma Family of Tumors in African-Americans

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**Background:** Ewing sarcoma family of tumors (ESFT) represent a spectrum of aggressive pediatric neoplasms, including Ewing sarcoma, peripheral primitive neuroectodermal tumor, and Askin tumor. All share recurrent translocations resulting in the fusion of EWS (22q12) with various members of the ETS family of transcription factors, predominantly FLI1 and ERG. Most of our knowledge base relating to ESFT is derived from the Caucasian population. The incidence of ESFT in African-Americans (AA) is low, approximately 1-5%. Subsequently, it's been difficult to determine whether AA-ESFT exhibit a similar biologic potential or harbor translocations involving the EWS gene locus akin to their counterparts in Caucasians.

**Design:** We retrospectively reviewed the clinical course and pathologic features of 13 AA children with ESFT treated at St Jude Children's Research Hospital (1978-2003). EWS translocation status was determined by RT-PCR (fresh tissue) and/or fluorescence in situ hybridization (formalin-fixed paraffin-embedded tissue). A dual color break-apart FISH probe cocktail (Vysis, Downers Grove, IL) was utilized, containing two large DNA probes flanking the EWS-R1 breakpoint region.

**Results:** The cohort included 6 males and 7 females, ages 4.5-18 years (median 13.7 years), and 6 with metastatic disease at diagnosis. All showed typical histomorphologic features of ESFT, with moderate to strong CD99 immunopositivity. Rearrangements involving the EWS locus were detected in 5 of 9 cases (56%) on which molecular analysis was performed. Four of these 5 patients had chest wall/rib primaries and all are dead of disease with a median survival of 9 months (range 8-17 months). The fifth patient had an extremity primary and is alive with no evidence of disease at 8 months from diagnosis. The 4 patients who had tumors with an intact EWS locus are alive with a median follow-up of 64 months (range 34-264 months). The association between EWS translocation status and outcome is statistically significant (p=0.016). We found no clear correlation between translocation status and age, stage, or size of lesion at diagnosis.

**Conclusions:** Our findings suggest that ESFT in AA may be separated into 2 distinct groups based on EWS translocation status: a "favorable" group with an intact EWS locus and prolonged survival, and an "unfavorable" group harboring EWS rearrangements and an aggressive clinical course.

### 1404 Myogenin and MyoD Expression in Pediatric Rhabdomyosarcoma (RMS) and Other Soft Tissue Tumors

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**Background:** Despite its distinct morphologic appearance in the classic alveolar and embryonal subtypes, pediatric RMS may exhibit a wide spectrum of morphologic variations making its diagnosis difficult. This is especially true for embryonal RMS, for which no diagnostic molecular abnormalities have been identified. Immunohistochemistry remains the main diagnostic tool in addition to the morphologic appearance of the tumor. Desmin and muscle specific actin have been traditionally used in the diagnosis of RMS, but their wide localization in tumors even in the absence of myogenous differentiation, has diminished their specificity. Myogenin and MyoD are products of muscle determination genes, which have been recently added to the panel of the diagnostic markers in RMS, because of their specificity for rhabdomyoblastic differentiation.

**Design:** In the present study we compared staining of 17 alveolar and 12 embryonal RMS with commercially available antibodies to myogenin (Novocastra Labs) and MyoD (DAKO). Because in a previous study myogenin staining was observed in a few non RMS spindle cell lesions, we included a panel of 33 non RMS tumors and tumor-like conditions for comparison. All tissues were formalin-fixed, paraffin-embedded and were obtained from the archives of the laboratory of Pathology at the NCI.
**Results:** We found that both myogenin and MyoD were present exclusively in RMS and tumors with rhabdomyoblastic differentiation, such as ectomesenchymomas and Triton tumor. Both antibodies yielded specific nuclear staining. Non specific cytoplasmic staining was observed only in 4 cases with the anti-MyoD antibody. From the 17 alveolar RMS, 13 were positive for both myogenin and MyoD and 4 were positive only for myogenin. From the 12 embryonal RMS, 4 were positive for both, 3 were positive only for MyoD, 3 were positive only for myogenin and 2 were negative for both markers. In general MyoD staining was more pronounced (higher intensity and percentage of positive cells) in the embryonal RMS.
**Conclusions:** Myogenin and MyoD are specific and sensitive markers in the diagnosis of pediatric RMS. MyoD is more sensitive in detecting embryonal RMS, whereas, myogenin is more sensitive in detecting alveolar RMS.

### 1405 Comparison of Tissue Microarrays Containing Bone Marrow Biopsies and Small Volume Cellular Aspirate Suspensions

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**Background:** Recently, gene expression profiling studies have been employed to investigate prognostic subgroups in pediatric acute leukemia. Tissue microarrays (TMA) are useful for high-throughput analysis of protein expression of target genes in acute leukemia samples and for validation of gene microarray analysis. We have recently demonstrated that small numbers of cells from bone marrow and fine needle aspirates can be constructed on a TMA. This method allows for effective study of tumor samples where sample size is often limited.
**Design:** A total of thirty-two (32) pediatric acute leukemia samples were studied. Previously frozen samples of bone marrow aspirates of 1 x $10^6$ - 1 x $10^7$ cells were spun, pellets placed in 1.0 ml eppendorf tubes, and fixed in formalin. Cores of 0.6 mm were taken from these tubes and directly embedded in a paraffin block to create the TMA. Additionally, 0.6 mm cores of accompanying paraffin embedded bone marrow biopsies were embedded in the same TMA block for comparison. A panel of eight (8) immunohistochemical (IHC) stains was performed.
**Results:**

Aspirates demonstrating positive staining

| | AML | pre-B ALL | T-ALL | Burkitt |
|---|---|---|---|---|
| n | 10 | 17 | 6 | 1 |
| CD79a | 0 | 15 | 0 | 1 |
| CD3 | 0 | 0 | 5 | 0 |
| MPO | 6 | 0 | 0 | 0 |
| CD163 | 3 | 0 | 0 | N/A |
| Tdt | 3 | 14 | 4 | 0 |
| CD34 | 1 | 0 | 0 | 0 |
| PAX-5 | 0 | 11 | 0 | 1 |
| CD10 | 0 | 13 | 3 | 1 |

N/A - No core present for evaluation; CD163 - Hemoglobin scanvenger receptor that detects monocytic differentiation

Biopsies demonstrating positive staining

| | AML | pre-B ALL | T-ALL | Burkitt |
|---|---|---|---|---|
| n | 3 | 12 | 3 | 1 |
| CD79a | 0 | 6 | 0 | 1 |
| CD3 | 0 | 0 | 3 | 0 |
| MPO | 2 | 0 | 0 | 0 |
| CD163 | 3 | 1 | 0 | 0 |
| Tdt | 0 | 9 | 3 | 0 |
| CD34 | 0 | 3 | 0 | 0 |
| PAX-5 | 0 | 5 | 0 | 1 |
| CD10 | 1 | 10 | 1 | 1 |

N/A - No core present for evaluation; CD163 - Hemoglobin scavenger receptor that detects monocytic differentiation

**Conclusions:** We show that IHC results from bone marrow aspirate pellets and bone marrow biopsies are comparable. This study confirms small numbers of cells can be utilized to construct TMAs for protein expression studies. The use of TMAs allows for analysis of multiple cases simultaneously. This technique can be effectively used in the future to analyze novel markers of diagnostic or prognostic importance in pediatric lymphomas / leukemias.

### 1406 Telomerase Expression in Primary, Metastatic and Post-Treatment Osteoblastic Osteosarcoma in Pediatrics

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**Background:** The proliferative capacity of cells is limited by the gradual loss of tandem nucleotide repeats (telomeres, TTAGGG) at chromsomal ends. Malignant cells may become immortalized if telomeric loss is compensated for by "reactivation " of telomerase. hTert (human telomerase reverse transcriptase) is capable of synthesizing the telomeric ends during each cell division. hTert reactivation has been associated with cellular immortalization.
**Design:** 15 children and adolescents with osteoblastic osteosarcomas of long bones comprised the study population. Formalin-fixed, paraffin-embedded tissues from the primary tumor biopsies (n=15), metastatic lung tumor biopsies (n=6) and tumor resection (n=15) following oncologic management were available for telomerase immunocytochemical analysis (anti-hTERT antibody, 1:200, Santa Cruz Biotechnology). hTert expression was graded based upon the proportion of tumor cells that were immunoreactive with hTert antibody (trace <1%; 1+ = 1 to 25%; 2+ = 26 to 50%; 3+ = 51 to 75%; 4+ = >76%).
**Results:** The study population was comprised of 9 females and 6 males. The mean age at diagnosis was 13.7 years (range 4 to 18 years). The tumors involved femurs (73%) and tibias (27%). Metastatic lung tumors were present in 40% of cases at initial diagnosis. hTert was detected by immunocytochemical analysis in all tumors. The majority of primary tumor biopsies had hTert expression grades of 3+ (5/15) or 4+ (7/15) with the remaining 3 tumors possessing an hTert expression grade of 2+. In contrast, normal bone from these cases was negative for hTert. hTert expression for metastatic lung tumors were either 3+ (2/6) or 4+ (4/6). Following oncologic management, hTert expression in viable tumor cells was trace (1/15), 1+ (5/15), 2+ (7/15) and 3+ (2/15). The mean hTert expression was 3.2 for the primary tumor biopsies, 3.7 for metastatic lung tumor biopsies and 1.7 for tumor resections following oncologic management
**Conclusions:** Telomerase activity (hTert) is expressed to a high degree in pediatric osteoblastic osteosarcomas within primary tumors and metastatic lung tumors. There is a trend toward higher expression in metastatic tumor when compared with tumor cells at the primary site. Following oncologic management, there is a dramatic decrease in telomerase expression with residual viable tumor cells within the resected tumor. Telomerase appears to participate in the neoplastic process associated with osteoblastic osteosarcoma and may represent a novel therapeutic target.

### 1407 Neuroblastoma Cell Type Heterogeneity Defined by Genomic and Proteomic Methods

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**Background:** Neuroblastoma is a neural crest-derived malignancy responsible for 15% of childhood cancer deaths, and often resistant to treatment. It is thought to be composed of at least three cell populations - an aggressive neuroblastic component resembling primitive neural cells, a benign-acting component resembling Schwannian stroma, and a "stem cell"-like component. Chemotherapy can cure some neuroblastomas, but it also causes a subset of tumors to progress/recur as predominantly neuroblastic tumors. *In vitro*, cell lines derived from primary tumors recapitulate phenotypic features of neuroblasts (N-type), Schwannian stroma (S-type), or both, and can spontaneously convert between these types. This provides a model system for studying the gene and protein expression profiles which distinguish these lines.
**Design:** We probed a Affymetrix microarray chip with RNA from cultured cell lines to identify genes differentially expressed between N- and S-type cells. Candidate genes revealed by this method were verified by immunohistochemical staining of tissue microarrays constructed from over 100 primary neuroblastoma tumors, cultured cell lines, and other neural and non-neural tumors. This methodology was used to simultaneously characterize expression in terms of overall level, subcellular localization, and neuroblastic versus stromal localization.
**Results:** Principal component analysis of gene expression data allowed N- and S-type cell lines to be easily distinguished. Tumor data analyzed according to this approach showed that tumors segregated according to neuroblastic and stromal content, which also correlated with clinical behavior. Analysis of gene expression showed that 75 genes were ≥5-fold upregulated in N-type cells ($p<0.01$) and 365 genes were ≥5-fold upregulated in S-type cells. Immunohistochemistry confirmed the differential expression of 20 genes highly specific for N- or S-type cells, as well as 25 genes of particular interest.
**Conclusions:** These results provide insight into new molecular pathways that may be involved in neuroblastoma tumorigenesis and could potentially serve as novel therapeutic targets. Knowledge of the genetic signature of subpopulations of neuroblastoma cells will foster a better understanding of their *in vivo* behavior, particularly with respect to predicting chemotherapeutic response and clinical outcome.

### 1408 EGFR and ERBB2 Protein Expression and Gene Amplification Status in Ewing Sarcoma Family of Tumors

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**Background:** The *EGFR* and *ERBB2* genes encode the transmembrane tyrosine kinases epidermal growth factor receptor (EGFR) and ERBB2, respectively, belonging to the epidermal growth factor receptor family. Amplification of either gene is associated with worse clinical outcome in certain tumor types, and both proteins are molecular targets of cancer therapy. In this study, we assessed the amplification status of the *EGFR* and *ERBB2* genes, surveyed expression of their protein products, and correlated our findings with clinical outcome in Ewing Sarcoma Family of Tumors.
**Design:** Twenty three formalin-fixed paraffin-embedded pretreatment tumors with morphologic and immunophenotypic features of ESFT were utilized to construct a duplicate-core tissue microarray. Fluorescence in situ hybridization (FISH) was performed using a dual-color break-apart probe cocktail (Vysis, Downers Grove, IL) flanking the *EWS*-R1 breakpoint region (22q12) and locus-specific probes targeting *EGFR* (7p12) and *ERBB2* (17q21.1). Immunohistochemistry was performed using antibodies against EGFR and ERBB2, and a positive reaction was defined as staining in more than 20% of tumor cells. Clinical data was available on 19 patients, with a median follow-up of 4.5 years (range, 0.6-23.1 years).
**Results:** Demonstrable rearrangements involving the *EWS* gene locus, as evidenced by red and green signal separation using the *EWS* break-apart probe set, were identified in all 23 tested cases. None of the cases were found to harbor amplifications of *EGFR* or *ERBB2* by FISH analysis. EGFR expression was identified in 1/23 (4%) and ERBB2 in 6/23 (26%) cases; co-expression of EGFR and ERBB2 was not encountered. Patients were 6 females (32%) and 13 males (68%) with a median age of 13.7 years (range, 2.6-

19.9 years). Eight (35%) patients had metastatic disease. Expression of EGFR or ERBB2 did not correlate with clinical outcome in this group of patients.
**Conclusions:** ERBB2 expression is more common than EGFR expression in ESFT. Expression of both protein products does not appear to be secondary to *EGFR* or *ERBB2* gene amplification or to correlate with clinical outcome.

### 1409 Global Gene Expression Profiling of Alveolar and Embryonal Rhabdomyosarcomas

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**Background:** The major types of pediatric rhabdomyosarcomas (RMS), embryonal (ERMS) and alveolar (ARMS), are associated with distinct clinical behaviors and require different treatment regimens. Accurate classification in difficult cases is facilitated by molecular diagnostic detection of the specific gene fusions present in the great majority of ARMS (*PAX3-FKHR* or *PAX7-FKHR)*. However, material for molecular diagnostic studies is not always available and there is a continuing need for immunohistochemical (IHC) markers to aid in making this distinction. Our aim is to identify new differential diagnostic markers by comparing the global gene expression profiles of ARMS to ERMS and of *PAX3-FKHR*-positive ARMS to *PAX7-FKHR*-positive ARMS.
**Design:** Gene expression profiles were obtained from 38 tumors (23 ARMS and 15 ERMS) using Affymetrix HG-U133A GeneChip microarrays containing 22216 probe sets (querying 18500 transcripts from about 14500 genes). All ARMS were fusion transcript positive (16 *PAX3-FKHR*, 7 *PAX7-FKHR*) and all ERMS were confirmed to lack either gene fusion. Unsupervised hierarchical clustering analysis was performed using data from all probe sets. Differentially expressed genes were identified by two tailed t-tests with Bonferroni correction.
**Results:** Most ARMS (20/23) and most ERMS (13/15) were well clustered on homogeneous branches of the hierarchical clustering dendrogram. Five tumors (3 ARMS and 2 ERMS) were poorly clustered on orphan branches. Using a stringent cutoff of Bonferroni-adjusted $p<0.01$ and at least 3-fold difference in expression, there were 36 probe sets that were differentially overexpressed in ARMS compared to ERMS, and 1 probe set differentially overexpressed in *PAX7-FKHR*-positive ARMS compared to *PAX3-FKHR*-positive ARMS. For instance, probe sets corresponding to cannabinoid receptor and sarcosine oxidase (among others) were remarkably overexpressed in ARMS relative to ERMS. Using the same criteria, no genes were significantly overexpressed in ERMS relative to ARMS.
**Conclusions:** The gene expression signatures of pediatric RMS correlate well with their histological and molecular subtypes. Microarray-based profiling reveals genes that are highly differentially expressed in specific histological and molecular subtypes of RMS, providing new leads in the search for IHC markers useful in differential diagnosis and prognostic stratification.

### 1410 Assessment of N-myc Amplification: A Comparison of FISH, Lightcycler PCR Monoplexing, and Traditional Blotting Methods Used with Formalin-Fixed, Paraffin-Embedded Neuroblastomas

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**Background:** Neuroblastoma is one of the more common solid tumors occurring in children. N-myc amplification in neuroblastoma remains as one of the most powerful prognostic indictors and is associated with a poor prognosis. Analysis of N-myc amplification is usually determined by Southern blot. However, this technology is cumbersome for routine clinical use which has lead to the development of Fluorescence in Situ Hybridization (FISH) as an alternative. However, FISH is time consuming and subjective. Because of these problems, we developed a novel, quantitative polymerase chain reaction (PCR) assay for N-myc amplification. In this report we compare our PCR N-myc assay with results obtained from Southern blot and FISH.
**Design:** Eighteen cases of neuroblastoma and twenty normal tissues were retrieved from pathology records at the Children's Hospital of Los Angeles and at the University of Utah Health Sciences Center. The N-myc amplification status was obtained from patient records, and recorded either as amplified or non-amplified. N-myc FISH was performed with the use of a commericial kit (Vysis Inc.). Quantitative polymerase chain reaction (PCR) was done with a monoplex reaction in which the amplification of N-myc is determined relative to a control gene; eukaryotic translation initiaion factor (IF2). Cases which showed an N-myc to IF2 ration of greater than 2.15 (three standard deviations above the mean of unamplifed tissues) were considered amplified.
**Results:** All of the Southern blot N-myc amplified cases were found to be amplified by both FISH and PCR. The FISH amplification results ranged from 10.9 to 20.4 and the PCR amplification results ranged from 14.7 to 1336. One of the Southern blot non-amplifed cases was found to be amplified by FISH. However, this case was difficult to evaluate because of high background. It was found to be negative by PCR. One non-amplifed Southern blot case was found to be amplified by PCR. However, this case was at a low level (2.3) right at the cut-off between amplified and non-amplified tissue (2.15). None of the normal tissues showed N-myc amplification by either FISH or monoplex PCR.
**Conclusions:** Monoplex PCR represents a novel method for quantitating N-myc amplification in neuroblastoma. Unlike Southern blot or FISH, it is linear and can identify tumors with greater than 1000-fold amplification

### 1411 Immunohistochemical Expression of Erythropoietin (EPO) and Erythropoietin Receptor (EPO-R) in Pediatric Neoplasms

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**Background:** Epo and EPO-R expressed on various nonerythroid tissues , are related to neural development and angiogenesis. Previous reports have described expression of EPO and functional EPO-R in adult human solid tumors of breast, ovary and uterus, as well as in pediatric tumor cell lines and some pediatric primary tumors of diverse lineages. The purpose of our study was to confirm the degree of expression of such biologic markers in a series of pediatric round cell tumors.
**Design:** Paraffin embedded sections from 30 cases of Ewing tumors, 40 cases of neuroblastoma (10 undifferentiated neuroblastomas, 10 poorly differentiated neuroblastomas, 10 Ganglioneuroblastomas and 10 Ganglioneuromas), and 10 cases of Rhabdomyosarcoma (5 alveolar and 5 embryonal rhabdomyosarcomas), were processed for immunohistochemical analysis following the streptavidin-biotin method and antigen retrieval (0.1M cytrate buffer pH 6.0). Sections were incubated with primary antibodies against EPO (goat polyclonal), and EPO-R (rabbit polyclonal), from Santa Cruz Biotech, Ca, USA.
**Results:** A diffuse cytoplasmic immunostaining was observed for EPO, whereas the immunoprofile of EPO-R was mainly membranous-cytoplasmic. Coexpression of both markers was evident in 20/30 Ewing tumors, 6/10 rhabdomyosarcomas and 35/40 cases of neuroblastic tumors. High expression (+++) of EPO and EPO-R was observed in the ganglion cells from GNB and maturing-mature GN. However, in the group of Ewing's tumors, no such overexpression was correlated with the degree of neuroectodermal differention.
**Conclusions:** EPO and EPO-R are molecules which are highly expressed in solid pediatric neoplasms. This expression correlates with the degree of neuronal maturation/differentiation within the group of neuroblastic tumors. EPO antagonists or agents that induce blockade of EPO-R signaling have to be evaluated in order to incorporate them as therapeutic modality in pediatric tumors.
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### 1412 IGF1R Blockade with a Novel Specific Small Molecule. A Potential New Strategy for Ewing Tumor Treatment

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**Background:** Ewing tumor (ET) cell survival and proliferation depends on several major autocrine loops. Targeting these autocrine loops is a promising therapeutical approach. We recently showed the cytostatic role of imatinib, a specific inhibitor of the SCF-KIT loop, in ET cells.
**Design:** We analyzed the *in vitro* impact of IGF1R blockade by ADW742, a small molecule specific for this receptor, in ET cell lines, alone and in combination with imatinib. We studied the impact on proliferation, apoptosis, cell cycle, pathway phosphorylation, colony formation, and motility of ET cell lines.
**Results:** IGF1R expression was detected in all ET cell lines by IHC, RT-PCR and/or western blot analysis. IGF1R expression was stronger in ET cell lines showing *EWS-FLI1* no-type 1 fusions, usually associated to a worse prognosis. Constitutive and ligand-inducible phosphorylation of IGF1R was found in all ET cell lines, indicating an active receptor. Treatment with IGF1R tyrosine kinase inhibitor ADW742 (0.25-15 μM) down-regulated IGF1R phosphorylation. Both AKT and MAPK pathways were inhibited. Dose-response inhibition of cell proliferation was observed ($IC_{50}$: 5-6 μM) up to 80%. ADW742 administered at similar doses induced an increase in apoptosis between 20-25%. The compound also blocked cell cycle progression reducing the number of cells in the S phase and blocking G1-S phase transition. Addition of imatinib to ADW742 further decreased the proliferative rate of ET cells between 20 and 50%, and increased the apoptotic rate of ET cells in 20-40%, suggesting the possible benefits of combined therapy. Interestingly, $IC_{50}$ of phosphorylation of AKT dramatically falled to less than 0.25 μM in combined treatments (5μM with ADW742 alone). Along with this data, combined therapy inhibited colony formation —reducing colonies in number and size—, and mobility of ET cells, inducing a change in ES cell morphology. These results show that treated ES cells acquire a much less aggressive phenotype.
**Conclusions:** Inhibition of IGF1 receptor mediated signaling can be effectively blocked by ADW742 in ET. The combination of ADW742 with Imatinib induces a significant reduction of tumor cell growth and increase in apoptosis, mainly by blocking both IGF1R-AKT, and KIT-MAPK pathways. Combined therapy also interferes with colony formation and mobility. This study supports a potential role for ADW742 in the treatment of Ewing tumor, alone or in combination, especially in patients carrying non-Type 1 fusions.

### 1413 Use of FISH on Paraffin-Embedded Tissues as an Adjunct to Diagnosis of Alveolar Rhabdomyosarcoma (ARMS)

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**Background:** Most RMS fusion gene type studies have been based on RT-PCR detection of the *PAX3-* or *PAX7-FKHR* fusion transcripts, a technique limited by RNA quality and failure of devised primer sets to detect unusual variants. As an alternative approach, we developed a FISH assay that can: (1) distinguish between the two most common ARMS fusion genes; (2) identify unusual variants; (3) assess individual histologic components in mixed ARMS/ERMS; and (4) be performed on paraffin-embedded tissue.
**Design:** Probe cocktails of BAC, PAC and cosmid clones were developed to detect rearrangements of *PAX3*, *PAX7*, and *FKHR* loci. After establishing the specificity of the probe sets on t(1;13) and t(2;13) positive cell lines and normal lymphocytes, we

evaluated touch preparations or paraffin-embedded sections of 68 specimens (35 ARMS, 14 ERMS, 6 mixed ARMS/ERMS, 11 non-RMS tumors, and 2 normal skeletal muscle specimens) using bicolor FISH. The presence or absence of t(1;13) or t(2;13) was confirmed by RT-PCR or cytogenetic analysis in 52/66 successfully FISH analyzed cases.

**Results:** Among all specimens with informative results for both FISH and RT-PCR or cytogenetics, *PAX-FKHR* classification results (*i.e.*, positive or negative) were concordant in 96.2% (50/52). The 2 discordant cases included one case exhibiting a t(2;13) by FISH that was later confirmed by repeat RT-PCR analysis and a second case with a rearrangement of the *PAX3* locus only (a result consistent with the presence of a *PAX3* variant translocation). Both ARMS and ERMS components of the mixed histologic subtype were negative for *PAX* and *FKHR* rearrangements, a finding confirmed by RT-PCR analysis or conventional karyotyping. Only 2 cases were not successfully analyzed by FISH, results possibly explained by prolonged formalin fixation.

**Conclusions:** These data: 1) demonstrate that FISH with these newly designed probe sets is a reliable and highly specific method of detecting t(1;13) and t(2;13) in routinely processed tissue (including paraffin-embedded) and may be useful in differentiating ARMS from other small round cell neoplasms, 2) suggest that FISH may be a more sensitive assay than RT-PCR in some settings (capable of revealing variant translocations), and 3) show that both alveolar and embryonal components of mixed ARMS/ERMS are negative for *PAX3, PAX7* and *FKHR* rearrangements.

### 1414 Terminal Deoxynucleotidyl Transferase-Positive Cells in Spleen, Appendix, and Branchial Cleft Cysts in Pediatric Patients

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**Background:** Terminal deoxynucleotidyl transferase (TdT) enzyme activity plays an important role in the development of lymphoid precursor cells and in the generation of T-cell receptor diversity. Until recently, it had been thought that the location of TdT+ lymphoid precursor cells was restricted mainly to the thymus and bone marrow. Recently, it was shown that TdT+ cells are present in significant numbers in both tonsils and reactive lymph nodes. Investigating the anatomic distribution and density of TdT+ precursor cells, we chose to evaluate spleen, appendix and branchial cleft cysts (BrCC) for the presence of TdT+ cells in pediatric patients, a group for whom a high frequency of TdT expression has been previously reported.

**Design:** H&E sections and TdT stains were evaluated in: 1) spleens in pediatric patients (n=27), 2) appendices (either acute appendicitis or incidental removal) (n=10) or 3) BrCC (n=7). All selected cases were in patients less than 18 years old. A small sample of spleen specimens (n=11) in adults with benign conditions were selected for comparison. Dual label immunohistochemical staining (TdT/CD79a, TdT/CD10 and TdT/CD3) was attempted on 7 cases which had the largest numbers of TdT-positive cells.

**Results:** In spleen, appendix and BrCC the range of TdT+ was 0-13, 0-96 and 0-6 TdT+ cells/hpf, respectively. One "BrCC" was identified as a thymic cyst. Although not uniform, the highest numbers of TdT+ cells were present in the youngest patients. In general, there were two patterns seen in spleens: scattered TdT+ cells in the PALS region of the white pulp, or widely scattered TdT+ cells in the red pulp. Dual labeling was marginally successful. Only rare colocalization of CD10/TdT and CD79a/TdT was noted.

**Conclusions:** We identified TdT+ cells in spleen, tonsil and BrCC, at varying levels of frequency. In pediatric spleens, the numbers of TdT+ cells are relatively low but appear increased in some reactive conditions, notably autoimmune processes or processes of immune stimulation. The literature suggests that increased numbers of precursor cells seen in the tonsil are a result of increased antigenic stimulation which may also account for increased TdT+ cells in appendix. This may reflect an increased influx of TdT+ cells as a result of higher antigenic stimulation at this site in comparison to spleen.

### 1415 Gastrointestinal Stromal Tumors (GISTs) in Children and Young Adults. A Clinicopathologic, Molecular and Genomic Study of Sixteen Cases

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**Background:** GISTs are mesenchymal tumors that typically present in adults over the age of 40 years. GISTs in younger patients are rare and not well characterized. Our objective was to define the pathologic and molecular characteristics of GISTs in children and young (less than 30 years old) adults.

**Design:** Clinicopathologic and molecular features, including *KIT/PDGFRA* genotype, in sporadic GISTs from 6 children and 10 young adults were analyzed. Gene expression analysis was performed in 7 gastric tumor samples from 2 children and 2 young adults, using a U133A Affymetrix platform and compared with 10 adult gastric GISTs.

**Results:** All 6 pediatric GISTs occurred in females, involved the stomach as multiple nodules, showed predominantly an epithelioid morphology, often involved lymph nodes (LN), and lacked *KIT* or *PDGFRA* mutations. Although all 5 patients for whom clinical follow up was available developed recurrence, 4 are still alive with disease after a mean follow-up of 85.2 months. Of the 10 young adults GISTs, 5 occurred in the small bowel and had spindle cell morphology, and 1 showed LN metastasis. *KIT* mutations were identified in 7 cases, 4 in exon 11 and 3 in exon 9. Seven patients developed recurrence and at last follow-up 2 patients have died of disease. Gene expression analysis showed high expression of *PHKA1, FZD2, NLGN4,* and *ANK3* in the pediatric versus older adult cases.

**Conclusions:** GISTs that occur in children represent a distinct clinicopathologic and molecular subset, with predilection for females, multifocal gastric location, and wild-type *KIT/PDGFRA* genotype. In contrast, GISTs in young adults represent a more heterogeneous group, including cases resembling either pediatric or older adult-type tumors. The distinct gene expression profile suggests avenues for investigation of pathogenesis and potential therapeutic strategies.

### 1416 Maternal Vasculopathy and Diagnosis of Pre-Eclampsia: Histological Analyses of Placentas

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**Background:** Pre-eclampsia or pregnancy induced hypertension (PIH) is characterized by hypertension, edema and proteinuria. It affects approximately 7% of pregnancies and usually occurs after 20 weeks of gestation. There are known pathologic changes that affect the placentas in PIH pregnancies, but these changes are not present in all PIH placentas. Two types of maternal vasculopathy have been reported which are believed to be pathognomonic to PIH: maternal vascular atherosis, characterized by infiltration of foamy macrophages within the vascular wall; and fibrinoid arterial medial necrosis, characterized by the presence of deep eosinophilic material within the maternal vessels.

**Design:** We investigated 350 placentas over a two and a half year period (January 2002-June 2004) with a hope to identify the potential usefulness of these changes in histologic diagnosis and corroboration of clinical pre-eclampsia. This study was carried out at West Virginia University Ruby Memorial Hospital, where 1271 placentas were examined macroscopically and microscopically for the period. The presence or absence of vascular changes, infarctions, thrombosis, abruption, chorangiosis and chorioamnionitis were investigated for each placenta with the clinical diagnosis of pre-eclampsia or PIH.

**Results:** The two types of vascular changes were found in approximately 21.4% (75/350) of placentas. These vascular changes were found mainly in the decidua capsularis of the membrane rolls, not on the decidua basalis of the maternal floor. There appeared to be no correlation of these vascular changes with placental weight, parity, and gestational age. There were more placental infarctions within these placentas (32.3%, 113/350). Intervillous thrombosis was 18.9% (66/350), microscopic abruption 6.3% (22/350), chorangiosis 10.9% (38/350), chorioamnionitis 8.6% (30/350).

**Conclusions:** These findings demonstrate the maternal vascular changes characteristic of pre-eclampsia are found in approximately 1 out of 5 pre-eclamptic placentas. Examination of pre-eclamptic placentas should be based on a combination of a number of histologic changes. Furthermore, the decidual capsularis of the fetal membrane roll, rather than the decidua basalis of the maternal floor of the placenta should be examined for these maternal vascular changes.

### 1417 Antiphospholipid Syndrome and Placental Deposition of Complement C4d

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**Background:** Antiphospholipid syndrome (APS) is associated with recurrent fetal loss, intrauterine growth restriction (IUGR), and characteristic placental pathologic changes. The etiologic mechanisms that result in antiphospholipid (aPL) antibody-mediated fetal tissue injury are complex and poorly understood. Studies utilizing a murine model of APS have demostrated a critical role of complement activation in leading to placental injury, fetal loss, and growth restriction in the presence of aPL antibodies. We examine the placentas of patients with APS for the presence of complement deposition to determine if the same mechanisms play a role in humans as well as mice.

**Design:** Immunohistochemical stain for C4d, a split-product generated by the classical pathway of complement activation, was performed on paraffin tissue sections of 36 patients with aPL antibodies and 9 normal control patients with no recognized placental pathology. A quantitative assessment of moderate to intense C4d staining quality is reported as none to minimal (0), moderate (1), and extensive (2). Areas of weak staining quality are not reported. Staining of extravillous trophoblasts (EVT), villous trophoblasts (VT) and the basement membrane of villous trophoblasts (BMVT) are individually scored. Results from controls and cases were compared using the chi square test.

**Results:** Immunoreactivity to C4d in the VT and BMVT was significantly stronger in the aPL antibody cases than in normal controls ($\chi^2 = 10.57$, $P = 0.005$ and $\chi^2 = 7.72$, $P = 0.02$, respectively). A significant difference was not present between cases and controls in C4d staining of EVT ($\chi^2 = 3.94$, $P = 0.14$).

**Conclusions:** We demonstrate a significantly greater immunoreactivity to C4d protein in the placentas of patients with APS compared to controls, which has not previously been demonstrated in humans. This finding suggests that placental activation of complement in APS plays at least a partial role in the etiologic mechanism of the poor reproductive outcome seen in these patients.

### 1418 Absence of Expression of hSNF5/INI1 in Malignant Rhabdoid Tumors of the Brain, Kidney and Soft Tissue: An Immunohistochemical Study with Implications for Diagnosis

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**Background:** Malignant rhabdoid tumors (MRTs) are highly aggressive neoplasms that usually occur in the brain or kidneys of children. The diagnosis of MRT depends on identification of characteristic rhabdoid cells — large cells with eccentrically located nuclei and paranuclear filamentous inclusions — and immunohistochemistry with antibodies to vimentin, keratin, epithelial membrane antigen and smooth muscle actin. In most MRTs, the hSNF5/INI1 gene located in chromosome band 22q11.2 is inactivated by deletion and/or mutation, so molecular confirmation is based on identification of an INI1 mutation.

**Design:** We assessed INI1 inactivation in 16 MRTs and 34 other tumors of the brain or kidney by immunohistochemistry using the BAF47/SNF5 antibody. Eleven brain, 3 renal and 2 soft tissue MRTs were examined along with 4 glioblastomas, 4 pilocytic astrocytomas, 4 oligodendrogliomas, 2 ependymomas, 2 choroid plexus papillomas, 5 pituitary adenomas, 4 germinomas, 4 renal cell carcinomas, 2 clear cell sarcomas, 2 Wilms' tumors and 1 medullary carcinoma.
**Results:** The neoplastic cells of all MRTs and the medullary carcinoma did not express INI1 consistent with inactivation of the gene. The neoplastic cells of all other tumors expressed INI1.
**Conclusions:** The findings suggest that INI1 inactivation occurs in a small subset of brain and kidney tumors that includes MRTs and, possibly, renal medullary carcinomas and that immunohistochemistry using BAF47 may be useful diagnostically.

### 1419 Cell Cycle Regulatory Proteins in the Podocyte Cell in Idiopathic Collapsing Glomerulopathy (CGN) in Children

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**Background:** The podocyte cell is a terminally committed cell in $G_1$ arrest of cell cycle. The cell cycle regulatory proteins (CCRP) are altered following podocyte injury and it is unable to overcome the $G_1$/S transition phase in children with minimal change disease (MCD) and classic focal segmental glomerulosclerosis (FSGS), in contrast to the dysregulated phenotype observed in adults with CGN (Kidney Int 2003:1374).
**Design:** Aim : To study the alterations in expression of cyclin dependent kinase inhibitors p27, p21 and p57, and cyclins D and A, in podocytes of children with CGN. 42 kidney biopsies were studied: MCD (14), FSGS (12), CGN (4) and normal (CON) (12). The sections were examined by dual staining immunohistochemistry. Podocytes were first identified by Wilm's tumor-1 staining and subsequently CCRP expression was analyzed. Statistical analysis was performed for the proportion of podocytes expressing each CCRP. ANOVA followed by Tukey HSD was used to compare the four groups.
**Results:** The podocyte expression for p27, p21, p57, cyclin D and A are shown in Table (mean ± SD).

| | CON | CGN | MCD | FSGS |
|---|---|---|---|---|
| p27 | 100±0.0 | 24.2±19.3 | 45.8±31.5 | 16.6±18.8 |
| CON vs | | <0.001 | <0.001 | <0.001 |
| CGN vs | | | NS | NS |
| p21 | 69.8±9.9 | 15.5±18.4 | 2.2±4.3 | 0.6±1.6 |
| CON vs | | <0.001 | <0.001 | <0.001 |
| CGN vs | | | 0.02 | 0.009 |
| p57 | 55.7±14.3 | 55.5±26.6 | 44.7±18.7 | 45.0±18.1 |
| CON vs | | NS | NS | NS |
| CGN vs | | | NS | NS |
| Cyclin D | 7.2±9.4 | 26.8±13.3 | 1.6±3.6 | 0.0±0.0 |
| CON vs | | <0.001 | NS | NS |
| CGN vs | | | <0.001 | <0.001 |
| Cyclin A | 0.0±0.0 | 10.3±6.7 | 0.0±0.0 | 0.0±0.0 |
| CON vs | | <0.001 | NS | NS |
| CGN vs | | | <0.001 | <0.001 |

p27 and p21 but not p57 was decreased in CGN, as in FSGS, compared to CON. Cyclins D and A were upregulated in CGN. The CCRP expression would suggest that podocyte cell in CGN is able to overcome the $G_1$/S transition phase. Thus the podocyte cell in CGN has the potential to proliferate in contrast to FSGS.
**Conclusions:** At the cellular level, changes in CCRP indicate the cell's response to injury. We propose based on the significant contrast observed in the podocyte cell's injury response between CGN (proliferative phenotype) and FSGS (non proliferative phenotype) that CGN should not be considered as a histological variant of FSGS.

### 1420 Quantitative Study of Topoisomerase Gene and Gene Product in Wilms Tumor Using FISH and IHC with Automated Imaging Analysis

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**Background:** Long-term survival rates for children with Wilms' tumor (WT) approach 90%, however prognosis for patients who relapse is only 30-50%. The goal of this study was to compare TOP2A gene copy numbers and TopoIIα protein expression by FISH and immunohistochemistry (IHC) with automated imaging analysis in a WT tissue microarray (TMA) to define prognostic parameters and mechanisms of WT drug resistance.
**Design:** Fifty seven WT cases (39 primary and 18 metastatic/relapsed and 12 normal kidneys) were subjected to TMA followed by IHC and FISH analysis. Nuclear expression of TopoIIα was quantified by using Chromavision Automated Cellular Imaging System (ACIS). To evaluate the TOP2A gene copy LSI TOP2A/CEP17 multi-color FISH was performed. Tumors with TOP2A/CEP17 ≥ 2 were amplified; ratios of 1.5-2.0 indicated gain and ratios ≤0.8 indicated deletion.
**Results:** TOP2A gene amplification/gain was detected only in 12% of WT. All these tumors belonged to patients with anaplastic tumors who died of disease progression. No deletions were found. Positive TopoIIα staining by IHC was observed in all 57 tumors. TopoIIα protein overexpression in WT was 51-fold as compared to non-cancerous adjacent kidney tissue ($p<0.001$). The average expression level of TopoIIα in primary WT was 17.9, whereas in metastatic and recurrent WT it was 24.1. 73% of patients did not have disease progression at 5 years if TopoIIα nuclear indices were below average (19.9) compared to 36% of those with TopoIIα levels above average. Higher TopoIIα protein expression levels were associated with higher tumor stage, anaplasia, development of metastasis ($p\leq0.05$), whereas decreased Topo IIα levels were associated with actinomycin D pre-op chemotherapy and better prognosis. The correlation between TOP2A/CEP17 ratios and TopoIIα protein expression levels in WT was weak ($r=0.292$). However TopoIIα nuclear indices were higher in all tumors with TOP2A gene amplification/gain: 27.8 vs WT mean value of 19.9.
**Conclusions:** Strong correlation between TopoIIα protein and mRNA levels, and lack of correlation between TOP2A gene copies and TopoIIα protein levels suggest that the abnormality responsible for elevated TOP2A gene expression is at the transcriptinal level in the majority of WT. TopoIIα protein expression by IHC may have a high prognostic value for predicting the responsiveness to TopoII inhibitors.

# Pulmonary

### 1421 DAX-1 and Androgen Receptor Expression in Diffuse Malignant Mesothelioma: Possible Targets of Hormonal Therapy

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**Background:** Diffuse malignant mesothelioma (DMM) is an aggressive malignancy of the pleura and peritoneum that invariably results in death of the patient. New approaches to therapy are needed for DMM. The nuclear receptor/ steroidogenesis regulator DAX-1 and androgen receptor (AR) are potential targets of hormonal or other pharmacological manipulations that might modify neoplastic growth. Expression of DAX-1 and AR by DMM has not been previously examined.
**Design:** A tissue microarray of 45 DMM cases was immunostained for DAX-1 (1:100 Santa Cruz Biotechnology, Santa Cruz, CA) and AR (1:40 BD Pharmingen, San Diego CA) using standard immunohistochemical techniques. Staining results were graded on a scale of 0-3 (0=no nuclear staining; 1=<33% of tumor cells; 2=33-66% of tumor cells; 3=>66% of tumor cells).
**Results:** All DMM were immunopositive for DAX-1. 13 DMM (30%) showed immunopositivity in 33-66% of cells and 32 (70%) showed expression in >66% of cells. 6 (13%) of DMM were negative for AR, 5 (12%) showed expression in <33% of cells, 10 (23%) showed expression in 33-66% of cells, 23 (52%) showed immunopositivity in >66% of cells.
**Conclusions:** The high frequency of DAX-1 and AR expression by DMM suggests that these receptors could represent possible targets of hormonal or anti-hormonal therapeutic agents. Additional studies exploring the effects of pharmacologic manipulation of DAX-1 and AR should be considered to more fully evaluate the therapeutic potential of this approach.

### 1422 Androgen Receptor Expression Correlates with Improved Survival in Early Stage Squamous Cell Lung Carcinomas: A High-Throughput Tissue Microarray Study

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**Background:** Androgen receptor (AR) immunonegativity has been associated with aggressive features in breast carcinoma, and AR plays a role in the development of prostate carcinoma. AR expression has not been previously investigated in NSCLC.
**Design:** High-throughput tissue microarrays containing 340 NSCLC with 5 years or more follow-up were immunostained with antisera to AR (1:40, BD Pharmingen, San Diego CA) using standard immunostaining techniques. For each sample, the percentage of NSCLC cells demonstrating nuclear AR staining was scored on a scale of 0-3 (0=no nuclear staining; 1=<33% positive; 2=33-66% positive; 3=>66% positive). Results were correlated with patient survival using Kaplan-Meier analysis.
**Results:** 299 NSCLC were stages I and II, and 61 were stages III and IV. Results of NSCLC staining for AR were as follows: 44 (13%) immunonegative; 14 (4%) = <33% positive; 31 (9%) = 33-66% positive; 251 (74%) = >66% positive. Nuclear staining with AR was closely associated with better 5-year survival in stage I and II squamous cell carcinomas ($p=0.02$). No statistically significant correlation was identified in other histologic types of NSCLC or in more advanced stage tumors.
**Conclusions:** AR-positive stage I and II squamous cell carcinomas have a significantly better prognosis than AR-negative stage I and II squamous cell carcinomas. This improved 5-year survival was not observed with other cell types.

### 1423 Intensity of Estrogen Receptor beta Expression Predicts Prognosis in Early Stage Pulmonary Adenocarcinoma

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**Background:** Estrogen receptor beta (ERb) has been identified in non-small cell lung cancers (NSCLC) and is a potential target for hormonal or other therapies. A study utilizing lung cancer cell lines showed significantly reduced cell proliferation in response to tamoxifen and slightly increased tumor cell growth with 17 beta-estradiol treatment, suggesting physiologic activity for this receptor. The prognostic significance of ERb in NSCLC, however, has not been investigated.
**Design:** Tissue microarrays consisting of 340 NSCLC with long-term follow-up of five or more years were immunostained with antisera against ERb (1:300, GeneTex, San Antonio, TX), using standard immunostaining techniques. The percentage of cells with