von Wasielewski R, Mengel M: Method for production of material blocks with multiple test samples. PCT International Publication Number: WO 01/51910 A1. 2001. International Patent Number: PCT/DE00/04647). To simplify this melting procedure by reducing it to one-step and to overcome restrictions of the MaxArray-technique concerning equipment and PTMA design we looked for a new technique.
Design: As recipient blocks (PTMAs) we used stabilization bodies (i.e. paraffinized biological material (e.g. liver tissue, lung tissue), blocks of paraffinized agar and synthetic spongy material (e.g. packaging material, sponges for cleaning)) cast in routine paraffin blocks. Prior to drilling the holes as presented previously, the later PTMAs were carefully trimmed in order to have the stabilization bodies reach the surface of the PTMA. After filling the holes with PTCBs, the PTMAs were put into routinely used steel embedding molds, put on a hot plate and heated up to 65°C. After fully melting the paraffin the steel embedding molds with the PTMAs were cooled down and processed routinely.
Results: The different materials used as stabilization bodies showed different properties at paraffinizing, drilling, melting and cutting. The best results were achieved with biological tissues. The folding and the detachment of the PTCBs was significantly reduced by creating a solid contact between the paraffins of the PTMA and the PTCBS. The stainings, especially the immunohistochemical reactions, did not show any deterioration due to the melting process. Well-known problems of paraffin sectioning like the disruption of sections were not solved by melting a PTMA after filling.
Conclusions: By using stabilization bodies a one step fully melting of the PTMAs is possible without the need for a special equipment and without any limitations concerning the number and the diameter of the holes of a PTMA.

1570 Depositing Archived Paraffin Tissue Core Biopsies in Paraffin Tissue Microarrays by Using a Paraffin Tissue Punch with a Countersunk

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Background: Paraffin tissue microarrays (PTMAs) as introduced by Kononen et al. in 1998 became a widely used technique in routine pathology and especially in research. By the use of a tissue puncher/arrayer (Beecher Instruments, USA), Kononen took paraffin tissue core biopsies (PTCBs) of 0.6 - 2 mm in diameter from routine paraffin tissue blocks and transferred them to another paraffin block with up to 1000 holes. However, till now archived PTCBs can not be used by the system of Kononen for the construction of PTMAs in contrast to the techniques as described by Wan et al. and Mengel et al.. In order to use archived PTCBs for PTMAs constructed with the most popular Beecher system we looked for a technique to overcome this drawback.
Design: We modified the commercially available Beecher paraffin tissue punch by applying a countersunk in the upper opening. To test this new punch PTCBs were punched out of routine paraffin tissue blocks and stored in Eppendorf tubes. Then PTMAs were constructed according to routine procedures. However, instead of punching the PTCBs, the PTCBs were transferred from the microtubes to the countersunk of the Beecher tissue punch using a tweezers. With the stylet, the PTCBs were further pushed into the punch and finally pressed into the holes of the PTMAs. After filling the holes, the PTMAs were cut and the sections processed according to routine procedures.
Results: We could construct PTMAs with up to 322 archived PTCBs. Seldom the PTCBs broke when they were handled with the tweezers or the stylet. The time to fill a PTMA with stored PTCBs was similar to PTMAs constructed with immediately punched and inserted PTCBs despite of the procedure of picking up the PTCBs with the tweezers.
Conclusions: By applying a countersunk to the tissue punch the insertion of archived PTCBs into the tissue punch is facilitated. Thus, the construction of PTMAs consisting of formerly archived PTCBs is possible by using the widely distributed Beecher System.

1571 Flow Cytometric Analysis of Vβ Repertoire for Assessing T-Cell Clonal Proliferations

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Background: T-cell lymphoproliferative disorders have features of T-cell subset restriction, aberrant CD4/CD8 ratio, antigen loss or inappropriate expression. Molecular analysis targeting T-cell receptor (TCR) gene rearrangements by PCR is commonly used to determine T-cell monoclonality. Flow cytometric assessment of TCR using TCR β-chain variable regions (TCR-Vβ) emerges as an alternative way to traditional molecular techniques.
Design: T-cell analyses were performed on sixty five patient specimens by conventional flow cytometry. TCRβ, γ and/or δ gene rearrangements (InVivoscribe Technologies, San Diego, CA) were assayed by PCR and ascertained using capillary gel electrophoresis. Selective patients with TCRαβ T-cell proliferations were evaluated for clonal Vβ repertoire. Flow cytometry for monoclonal TCR-Vβ expression were performed using IOTest Beta Mark TCR-Vβ Repertoire Kit (Beckman Coulter, Miami, FL). Cases chosen include T-ALL, T-cell large granular lymphocytic leukemia, hepatosplenic T-cell lymphoma and other T-cell lymphoproliferative disorders. Analyses were performed using 5-parameter, 4-color immunophenotyping with incorporation of CD3 gating. Clonal expansions were quantified with established criteria and examined with EXPO software (Beckman Coulter).
Results: Twenty-eight (43%) patient specimens demonstrated clonal gene rearrangements by PCR assays. Twenty specimens positive by PCR showed monoclonality with TCR-Vβ analyses by revealing a predominant Vβ subpopulation, or most T-cells nonreactive with any Vβ antibodies; other cases, however, demonstrated only suspicious or non-clonal flow cytometry results. Suspicious cases tended to have substantial restrictions of T-cell subpopulations. Non-clonal specimens by TCR-Vβ expression had very low abnormal circulating lymphocytes, below the threshold of detection. Two cases of reactive T-cell proliferation in autoimmune disorders showed clonal Vβ repertoire.
Conclusions: TCR-Vβ expression by flow cytometry serves as an attractive addition to multicolor, multiparameter immunophenotyping. It has advantages of rapid turnaround time, convenient T-cell subset studies and quantitative results. Even though less sensitive than PCR, this approach may be used as an initial screening for T-cell monoclonality, particularly in laboratories without immediate access to molecular analyses. The particular Vβ+ T-cells can be monitored during chemotherapy once that subpopulation is identified. The limitations may be the expenses and suboptimal sensitivity.

1572 Analysis of T-Cell Clonality Using Laser Capture Microdissection and High Resolution Microcapillary Electrophoresis

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Background: Polymerase chain reaction (PCR)-based analysis of T-cell receptor (TCR) gene rearrangements is a useful method in the diagnosis of lymphoproliferative disorders. Identification of a clonal lymphocytic population may be difficult because of the paucity of the infiltrate as well as a heterogeneous population of cells. The aim of this study was to assess the diagnostic utility of LCM and high resolution microcapillary electrophoresis in the clonality analysis of small biopsy specimens.
Design: Clonality was assessed in 24 cases, including five reactive tonsils, five reactive lymph nodes, six inflammatory skin lesions, and eight T-cell lymphomas (three nodal and five cutaneous). CD3 positive T-lymphocytes were captured by LCM (Arcturus) from paraffinized IHC stained sections. Genomic DNA was extracted from the microdissected cells as well as from the whole tissue sections and subsequently analyzed for TCR-γ gene rearrangement by PCR. PCR products were detected using high resolution microcapillary electrpophoresis with the DNA 500 LabChip and Agilent Bioanalyzer.
Results: For LCM, numbers of cells varying from 10-10,000 were captured. We determined that 10 captured cells were sufficient to obtain a PCR product in the TCR-γ gene rearrangement assay using non-nested PCR. TCR-γ gene rearrangement analysis revealed monoclonal bands when the cell number was between 10 to 1,000 cells in reactive tonsils, lymph nodes, and inflammatory skin infiltrates. This pattern changed to polyclonal when higher numbers of cells were microdissected (2,000 to 10,000 cells). In contrast, LCM captured lymphoma cells were constantly monoclonal whether low or high numbers of cells were microdissected. The monoclonal peaks from LCM were identical in base pair size to those from the whole section extracts. Microcapillary electrophoresis coupled with LCM provided improved diagnostic sensitivity. In two of eight lymphoma cases, LCM revealed diagnostic monoclonal bands, whereas routine TCR-γ assessment of whole tissue sections with 10% polyacrylamide gel electrophoresis demonstrated only minor clonal bands.
Conclusions: Clonality determined by LCM is cell number dependent. We conclude that biopsy specimens containing low number of reactive polyclonal T-cells (less than 2,000 cells) may produce pseudomonoclonal bands and these cases should be interpreted with great caution. LCM coupled with microcapillary electrophoresis results in higher sensitivity and facilitates TCR-γ gene rearrangement analysis.

Ultrastructural

1573 Pediatric and Adult Hepatic Embryonal Sarcoma: A Comparative Ultrastructural Study with Morphologic Correlations

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Background: Hepatic embryonal (undifferentiated) sarcoma (ES) is a rare pediatric tumor occurring predominantly in the first decade of life, but few examples of adult ES have been also described. Isolated ultrastructural reports describe contradictory lines of differentiation in these tumors. The objective of this study is to identify the distinctive ultrastructural features of ES in both pediatric and adult age groups and to correlate the findings with morphology.
Design: Four pediatric and 3 adult ES cases with available Electron Microscopic and Pathologic material were identified. The morphologic features analyzed included cell type, presence of giant cells, nuclear pleomorphism, cytoplasmic content, eosinophilic globules, necrosis, and presence of inflammation. The ultrastructural findings recorded were cell type, presence of secondary lysosomes with dense precipitates, RER and mitochondria morphology, intracytoplasmic fat droplets and cytoplasmic filaments.
Results: Morphologically, all cases showed plump to spindle cells, bizarre giant cells, eosinophilic globules and necrosis. In 5 cases areas of myxoid stroma and inflammatory cells were noted. Ultrastructurally, the hallmark features in all 7 cases included sequestered and dilated RERs and secondary lysosomes with dense precipitates. Dilated mitochondria were seen in 6/7 cases, and in 3 mitochondrial-RER complexes. Other features included intracytoplasmic fat droplets (5/7), scant actin microfilaments (3/7), and focal glycogen pools (2/7). In 4/7 cases, small undifferentiated cells with minimal amount of cytoplasm and cellular organelles were also identified.
Conclusions: Hepatic ES have distinctive ultrastructural findings, including dilated sequestered RER and dense lysosomal precipitates, which correlate with the eosinophilic hyaline bodies seen microscopically. These findings suggest that ES are composed of fibroblastic, fibrohistiocytic and undifferentiated cells. Other lines of differentiation were not identified in this series. The pediatric and adult ES show similar morphologic and ultrastructural features.

1574 Comparative Ultrastructural Analysis and *KIT/PDGFRA* Genotype in 125 GastroIntestinal Stromal Tumors (GIST)

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Background: GISTs are the most common mesenchymal neoplasms of the digestive tract and are thought to originate from or differentiate toward the interstitial cell of Cajal lineage. Almost all GISTs express KIT protein and the majority show activating mutations in either *KIT* or *PDGFRA* proto-oncogenes.Ultrastructurally, these tumors have been shown to have either a smooth muscle, neuronal, dual, or null phenotype. Our objective was to investigate the relationship between ultrastructural features and genotype in a large series of GISTs.

Design: 125 histologically confirmed, CD117 positive, GISTs, with adequate tissue for ultrastructural and molecular analysis, were selected. PCR analysis for the presence of *KIT* exon 9, 11, 13, and 17 and *PDGFRA* exon 12 and 18 mutations was performed. The pathologic (tumor location, morphologic type), ultrastructural (neurosecretory-type granules [NS-G], microtubules, actin microfilaments, skeinoid fibers, cell processes, etc) and molecular (the presence and type of *KIT/PDGFRA* mutations) features of these tumors were gathered independently and correlated for possible associations.

Results: There were 62 (50%) tumors located in the stomach, 45 (36%) in the small bowel, and 9 (14%) in other locations. Histologically, 22 (18%) were predominantly epithelioid, 100 (80%) had a spindled morphology and 3 (2%) showed mixed features. Overall, *KIT* mutations were detected in 93 (75%) patients: 86 (69%) in exon 11, 7 (6%) in exon 9, and none in exon 13 or 17. A *PDGFRA* mutation was detected in 7 (6%) cases and 25 (20%) cases had no mutation. Ultrastructurally, skeinoid fibres were seen in 55 (44%) cases. Focal actin microfilaments were identified in 82 (65%) cases, but was more pronounced in only 5 (4%) cases. Rare NS-G were seen in 34 (27%) of cases, but were seen in most of the cells in only 5 (4%) cases. 7 (6%) cases showed both NS-G and microtubules. Interdigitating cell processes were seen in 71 (57%) cases and were very long and slender in 7 (6%) cases.

Conclusions: GISTs showing both NS-G and microtubules were associated with *KIT* exon 11 genotype and spindle cell morphology. Presence of skeinoid fibers or actin microfilaments did not correlate with location or mutation type. In contrast, *KIT* exon 11 insertion GISTs were associated with gastric location, spindle cell morphology, and lacked skeinoid fibers. *PDGFRA* mutated cases were associated with gastric location, predominantly epithelioid morphology and lacked NS-G.

1575 Urothelial Carcinoma in Culture: Ultrastructural Characterization of 13 Cell Lines

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Background: The development and characterization of tumor cell lines is essential for the assessment of subsequent functional experiments. Tumor cells in culture may display some of the features that characterize their specific phenotype in vivo. Urothelial cells have a specialized, uneven apical membrane, with a thicker inner leaflet, and with plates and foldings. In addition, urothelial cells may show variable degrees of glandular or squamous differentiation. The purpose of this study was to analyze the ultrastructural features of 13 urothelial carcinoma (UC) cell lines, with particular emphasis on apical differentiation, in relationship to their genetic alterations.

Design: The 13 UC cell lines included in the study were: 5637, 253J, 575A, J82, JON, MGH-U3, MGH-U4, RT4, SW780, SW800, SW1710, VMCUB-1, and VMCUB-3. From these, 3 lines were derived from grade I, 2 from grade II, and 3 from grade III urothelial carcinomas, and 1 from a bladder adenocarcinoma. Cells were processed for electron microscopy on the culture plate, and re-embedded in a perpendicular orientation. Thin sections from two representative blocks were examined. *TP53* and *FGFR3* mutational status was determined in our laboratory in 12 of the cultures, and 6 each were wild type or had *TP53* mutations. All cells harboured wild type *FGFR3* sequences.

Results: In the apical domain, microvilli were usually moderately abundant, and they were short in 9 cell lines and long in 4. Plates and foldings were usually scanty and not present in all cells, but could be found in 12 cell lines. In addition, 7 cell lines each had prominent RER, abundant mitochondria or secondary lysosomes. Large glycogen pools were found in 2 cell lines and tonofilaments were prominent only in one. All these features were found in a similar proportion of wild type and *TP53*-mutated lines, and also in a similar distribution among the different grades.

Conclusions: Urothelial differentiation is preserved in most of the UC cell lines in this study. Short microvilli are usually prominent. Tonofilaments are absent in most cell lines. These features appear to be unrelated to the *TP53* mutational status or grade of the original tumors. Electron microscopy is a good ancillary tool in the assessment of UC cell line phenotypes, in particular in order to examine the effects of genetic manipulation in vitro.

1576 Response of Proliferative Class IV Lupus Nephritis Following Cyclophosphamide Therapy in Children and Adolescents: Clinicopathologic Study

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Background: Therapy for proliferative lupus nephritis (Class IV LN) remains controversial. Intravenous cyclophosphamide has been shown to improve long-term renal function in lupus patients. A pediatric clinicopathologic study has not been undertaken. Assessment of initial and post-therapy biopsies to determine therapeutic effect and guide further management has not been evaluated.

Design: From 1990 to 2004, 25 pediatric patients with Class IV LN underwent an initial biopsy followed by 9 monthly infusions of cyclophosphamide (500-750 mg/m^2) and then a repeat biopsy to assess therapy. WHO LN criteria were utilized. Glomerular filtration rate and proteinuria were determined before and after treatment. Renal biopsy tissue was available for light, immunofluorescent and electron microscopic study. Statistical analysis was performed (Chi-Square, Mann Whitney U test, Student's t-test)

Results: Following therapy, LN was assessed as Class II in 32% (8/25), Class III in 56% (14/25) and Class IV in 12% (3/25). The decrease in LN class was significant ($p<0.01$). Prior to therapy, most cases were diffuse proliferative (92%, 23/25), with the remaining 8% being segmental (2/25). Following therapy, there were a limited number of Class IV cases (3) and these had diffuse proliferative lesions ($p<0.03$). Crescents were found in 44% prior to treatment and in no cases following therapy ($p<0.003$). Prior to therapy, activity index indicated that 68% were active, 32% were active/chronic, and none were chronic. Following therapy (Class III and IV LN), the activity index was assessed as active in 53%, active/chronic in 29% and chronic in 18%. Class V LN was also present in 24% before treatment and in 32% after therapy. Glomerular filtration rate improved significantly ($p<0.001$) from 102 ml/min/1.73m^2 to 132 ml/min/1.73m^2. Proteinuria decreased significantly ($p<0.002$) from 3,248 mg/d to 627 mg/d. Chronic renal failure and end-stage renal disease occurred in only 4% (1/25, mean follow-up 3.5yr).

Conclusions: In a pediatric population, intravenous cyclophosphamide therapy provided significant improvement in renal function, and reduction in the histologic LN class. Renal biopsy at the end of therapy provides the clinician with critical information regarding the need for additional aggressive therapy (additional intravenous cyclophosphamide) or maintenance therapy.

1577 An Ultrastrucutral Morphometric Analysis of Laryngeal Epithelium in Laryngopharyngeal Reflux

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Background: It is generally recognized that gastroesophageal reflux disease (GERD) can be associated with ear, nose, and throat signs and symptoms, a condition often referred to as laryngopharyngeal reflux (LPR). However, the morphologic alterations of laryngeal mucosa associated with LPR are currently poorly understood. Since the dilation of intercellular spaces between squamous epithelial cells is considered a morphologic marker of acid damage to esophageal mucosa in GERD, we evaluated whether similar changes can be detected in the laryngeal epithelium of patients affected by LPR.

Design: The study group included 15 consecutive patients (14 males and 1 female, median age 58.7 years) affected by LPR and 7 normal controls, who underwent laryngeal biopsies at the interarytenoid area of the posterior larynx. Tissue fragments were routinely processed for electron microscopy. Morphometric analysis of the intercellular spaces was conducted using a computer aided image analysis system (Quantimet, Leica, Cambridge, UK). At least 100 measurements were performed in each case, with care to consider basal and suprabasal layers of the squamous epithelium. Statistical tests were performed using the SPSS software (release 13.0, SPSS Inc., Chicago, IL). The Mann-Whitney U test was employed to compare two groups of continuous values. A $P<0.05$ was considered significant.

Results: Ultrastructural analysis demonstrated an irregular intercellular space dilation in specimens from the group of patients with LPR. Another ultrastructural abnormality observed in a minority of patients was the presence of numerous cytoplasmic vacuoles. Computer assisted morphometric analysis demonstrated that the intercellular space distance between squamous cells was significantly wider in patients with LPR than in control subjects (411.7 nm ± 188.6 SD vs. 155.8 nm ± 56.4 SD, $p=0.003$).

Conclusions: These data indicate that ultrastructural evidence of dilation of the intercellular spaces of epithelial cells in the larynx may be a morphologic marker of acid reflux, as already described in esophageal mucosa. If this result will be confirmed in larger series, it may provide a useful diagnostic tool for the identification of LPR.

1578 Progressive Increase in Basal Cell Caveolae throughout the Stages of Induced Canine Prostatic Hyperplasia

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Background: Prostatic hyperplasia (BPH) is a very prevalent disease with a remarkable clinical impact. However, the pathogenesis of BPH is not well understood. In addition to man, BPH only occurs spontaneously in the dogs, where it can also be experimentally induced. The basal cell (BC) compartment contains the transient amplifying population; it is therefore conceivable that it plays a role in the development of BPH. In a previous study, caveolae were observed in BC. Among other roles, caveolae are considered sites for hormone receptor ligand interactions. The purpose of the present study was to determine, by means of ultrastructural morphometry, if the number of caveolae was related to the stages of hormonally induced BPH.

Design: Eight male beagle dogs were divided into two groups (G I, n=5; G II, n=3). G I dogs were castrated and injected with 5α-androstane-3α,17β-diol, and 17βestradiol for 38 weeks. G II dogs received only vehicle. Surgical prostate biopsies were obtained before castration without treatment, and then at 45 day intervals for 9 months, resulting in 7 experimental stages, M0 through M6, the latter again without hormonal treatment. Tissue was processed for electron microscopy. Electron micrographs were taken and caveolae per 100 µm of BC membrane counted. Data were processed by the SAS statistical package. All animals were treated in accordance to the UAB-Veterinary School's guidelines.

Results: Increasing numbers of caveolae in BC were noted from M0 (17%) and M1 (24.9%) to M2 (44.8%), M3 (41.3%), M4 (33%) and M5 (36.2%), these latter values suggesting a plateau. The difference between these four stages and the initial ones was statistically significant ($p<.05$). At M6 stage, after hormonal treatment withdrawal,

caveolae diminished dramatically in number (18.4%). These difference was also statistically significant (p<.05).
Conclusions: In this model of experimental BPH, the number of caveolae in BC significantly increases with the hormonal treatment, and markedly diminishes after hormonal withdrawal. This suggests that BC are crucial in the development of BPH and that they are either a primary target of or induced by hormonal stimuli. The role of caveolae in this disease seems to be very important and merits further research.

1579 The Ultra-Structural Spectrum of Intracytoplasmic Crystals Associated with B-Cell Lymphoproliferative Disorders

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Background: Crystals seen in B-cell proliferative disorders are deposits of monoclonal immunoglobin which precipitate due to possible structural defects and form crystalline inclusions. The crystals can demonstrate a spectrum of morphology. Crystals are not uncommonly seen in non-neoplastic and neoplastic disorders. Crystal deposition in organ systems other than the primary organ have been reported. It is important to recogonize crystals related to underlying malignancy and distinguish them from other non-specific inclusions which may be seen in unrelated or benign conditions. The aim of our study was to identify the mophologic spectrum of crystals seen on ultrastructal examination of B-cell proliferative disorders.
Design: We identified from our Electron-microscopy files 7 cases of B-cell proliferative disorders which showed crystal deposition. Immunofluorescence study was performed on 3 of the cases and immunogold labelling was performed on 2 of them, when the original material was available.
Results: Of the 7 cases 4 were chronic lymphocytic leukemia (CLL), 3 patients had underlying plasma cell dyscrasia. These crystals were seen in various forms, needle like, rhomboid, rectangular, cuboidal, polygonal with rounded ends. These crystals were found in the cytoplasm of plasma cells, B lymphocytes, most often within the confines of rough endoplasmic reticulum (RER) and Golgi apparatus. Rarely they were seen in the extracellular space admixed with fragments of cytoplasm. In one case the crystalline material was phagocytosed by histiocytes. Two renal biopsies showed rhomboid shaped crystals with a lattice like substructure in proximal renal tubules.Of these one had prior diagnosis of mutiple myeloma, and later developed AL amyloidosis and the other had presented with renal failure post analgesic nephropathy. This patient showed only monoclonal band (kappa) on serum electrophoresis (MGUS). The crystals were charcterized as kappa light chains by both immunofluorosence and immunogold labelling.
Conclusions: The chemical uniformity of the product secreted by the cells involved in the various types of monoclonal gammopathy, B-cell lymphomas and leukemias (gamma globulins or its constituents light and heavy chains) often results in formation of crystals which are best resolved by EM. These can deposit in other organ systems as well and produce symptoms related to dysfunction of these organs. It is important to recogonise and characterize these crystals as they may be the only clue to the underlying malignancy.

1580 Rothmund-Thomson Syndrome in Pediatric Osteosarcoma: Clinicopathologic Features

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Background: Rothmund-Thomson syndrome (RTS) is a rare autosomal recessive disorder, characterized by poikiloderma, skeletal anomalies, small stature, sparse hair, juvenile cataracts, and increased risk for developing osteosarcoma (OS). Recently, constitutional mutations in the *RECQL4* gene (encodes RECQ helicases) have been identified in about two-thirds of RTS patients. Genotype-phenotype analysis has shown a correlation between presence of truncating mutations and prevalence of OS in RTS.
Design: The purpose of this study was to characterize osteosarcomas in RTS patients with respect to age at diagnosis of OS, location of OS, histopathologic subtype of OS based upon histopathologic and ultrastructural features, histopathologic response to chemotherapy, rate of secondary malignancies and survival. Eleven RTS subjects diagnosed with 14 separate OS were identified (age range 4-20yrs, median age 9yrs, 6M:5F). Tissue was available for mutational analysis, and light and electron microscopic evaluation.
Results: OS sites of involvement were: distal femur (7), proximal tibia (3), proximal humerus (1), distal fibula (1), distal ulna (1), distal radius (1) and patella (1). OS histopathologic types were: Osteoblastic (10), fibroblastic (2), telangiectatic (1) and giant cell rich (1). Electron microscopic features were necessary to distinguish fibroblastic, telangiectatic and giant cell rich OS from other non-OS tumors. *RECQL4* mutations were identified in all OS tumors. Histopathologic response to chemotherapy was: Grade I (<50% necrosis) 4/9, Grade II (>50 to <90% necrosis) 1/9, Grade III (90-99% necrosis) 3/9 and Grade IV (100% necrosis) 1/9. Outcome was Alive with No Disease 5/9, Died of Disease 3/9, and Died of Secondary Malignancy 1/9.
Conclusions: OS in RTS occurs at a younger median age (9yr) than in the typical OS population (17-20yr). OS locations and histopathologic types are similar to that for the typical OS population. Ultrastructural examination is helpful in distinguishing fibroblastic, telangiectactic and giant cell rich OS from other non-OS tumors. RTS pateints with OS have similar responses to chemotherapy and similar outcomes as the typical OS population. Similarities in clinicopathologic features with RTS suggest that mutations in the *RECQL4* pathway participate in the pathogenesis of RTS and sporadic OS. *RECQL4* pathways are currently under investigation with sporadic OS.

1581 Rhabdomyoma-Like Rhabdomyosarcoma in a *PTCH* Heterozygote Murine Model

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Background: *PTCH* gene mutation is associated with Gorlin's syndrome and has been identified in sporadic and syndrome-associated rhabdomyosarcomas. A Gorlin's syndrome murine model has been developed (*PTCH* +/-). The resulting animals have a complex phenotype and may develop rhabdomyosarcomas, medulloblastomas, lymphomas, gastrointestinal stromal tumors, abdominal cysts and musculoskeletal abnormalities.
Design: Tumor and normal skeletal muscle tissues for gene microarray (Mouse 15K Microarray), RT-PCR, routine light microscopy, immunocytochemistry and electron microscopy were obtained from 20 *PTCH* +/- mice with rhabdomyoma-like rhabdomyosarcomas. Cardiac tissue from these animals, as well as adjacent nontumorous skeletal muscle, served as controls. Immunocytochemsitry evaluation included Osteopontin NFkB and matrix metalloproteinase 2 (MMP2).
Results: The histopathologic and ultrastructural features of the *PTCH* +/- tumors revealed a mixture of relatively well-developed neoplastic skeletal muscle cells and a range of rhabdomyoblastic cells. The tumor cells possessed thick and thin myofilaments with z-band material on electron microscopy. The tumors had a rhabdomyoma-like rhabdomyosarcoma appearance. Microarray analysis discovered 33 upregulated genes, in particular osteopontin associated with angiogenesis, cell adhesion and extracellular matrix production. Osteopontin activates NFkB signaling which in turn induces MMP-2. RT-PCR indicated a 4-fold increase in Osteopontin, a 6-fold increase in NFkB, and a 2-fold increase in MMP2 in tumors compared with controls. Immunocytochemistry demonstrated diffuse, intense expression of Osteopontin and NFkB with the tumor cells, and focal intense expression of MMP2 in tumor cells. Adjacent normal skeletal muscle demonstrated no expression of NFkB and MMP2, and only cell surface and interstitial Osteopontin expression.
Conclusions: Loss of heterozygosity or mutation in the *PTCH* gene may be mediated, in part, by Osteopontin upregulation, an integrin-binding phosphoglycoprotein and a transcriptional target for *TP53* and the oncogene *GLI*. A signaling cascasde that begins with *GLI* upregulation leading to overexpression of Osteopontin and subsequent activation of *NFkB* and *MMP2* by osteopontin participates in the neoplastic process with *PTCH* gene dysregulation in murine rhabdomyoma-like rhabdomyosarcoma. Growth regulator inhibitors may be therpeutic targets in rhabdomyosarcomas.

1582 Ultrastructural Changes in Prostatic Hyperplasia: A Comparative Study of Human and Experimentally Induced Canine Disease

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Background: Prostatic hyperplasia (BPH) is a very prevalent disease with a remarkable clinical impact. However, the pathogenesis of BPH is not well understood. In addition to man, BPH only occurs spontaneously in the dog, where it can also be experimentally induced. We report the results of a comparative study on ultrastructural findings from human and different stages of experimentally induced canine BPH.
Design: Samples of prostatic tissue for ultrastructural study were obtained from 12 cases of human BPH. These were compared to the samples from an experimental model of canine BPH: 8 beagle dogs (11/2-2 y.o.) were classified into two groups (G I, n = 5, and G II, n = 3). G I dogs were castrated and injected 25 mg of 5α-androstane-3α,17βdiol, and 0.25 mg 17βestradiol and G II only vehicle, for 38 weeks. Prostate biopsies were obtained before castration and without treatment, and at 45-day intervals for 9 months (7 experimental stages, M0-M6, the latter again without hormonal treatment). Tissue was processed for electron microscopy and examined under a Philips CM100 microscope.
Results: In different stages of canine BPH, luminal epithelial cells showed prominent, often dichotomized microvilli, apical granule sub-compartmentalization, and elongated shapes, with luminal cytoplasmic fronds. Basal cells contained increasing numbers of caveolae. These changes were dramatically reversed upon treatment withdrawal. In the human prostate, similar changes were observed, but they were usually of lower intensity. Overall, the stromal component was comparatively less prominent in canine than in human prostate samples.
Conclusions: Subtle, mostly apical changes occur in the luminal cells at the ultrastructural level in both canine and human BPH. In both species, the most prominent change in the basal cells is the presence of caveolae in their basal aspect. Primary epithelial changes are more difficult to assess in human BPH than in the canine experimental model, due to the superimposed alterations derived from stromal overgrowth in the former. Thus, the canine prostate model is optimal for the study of changes in epithelial cells in BPH.

1583 Gastrointestinal Stromal Tumors(GISTs): An Immunohistochemical and *Ultrastructural* Study on 17 Cases

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Background: Gastrointestinal stromal tumors (GISTs) are KIT-positive mesenchymal tumors in the GI tract and intra-abdominal soft tissues (EGISTs),which are considered to derive from the interstitial cells of Cajal (**ICC**).We evaluate **17 cases of GISTs** for a variety of immunohistochemial(IHC) and **ultrastructural features** to identify **site-specific similarities and differences**.

Design: The **17** patients'ages ranged from 40 to 79 years (mean:59).The tumors were located in the **stomach** (9),**duodenum** (3),**ileum** (1),**mesentery** (1),**omentum** (1),**retroperitoneum** (1),and **pelvic cavity** (1).H-E morphology,IHC study using KIT,CD34,vimentin,smooth muscle actin (SMA),S-100 protein,desmin and MIB-1,and EM study were performed.

Results: Histopathologically,the 9 gastric GISTs showed 6 spindle cell,1 epithelioid,and 2 mixed types.Three duodenal GISTs were 2 spindle cell and 1 mixed types,and all presented extracellular **skeinoid fibers**(SF).One ileum GIST was mixed type.Four EGISTs showed 1 spindle,1 epithelioid,and 2 mixed types. **Immunohistochemically**, KIT(+) in 17 cases,CD 34 in 14,vimentin in 17,SMA in 2,S-100 in 3.The MIB-1 index ranged from 3~67% (mean:19%).S-100(+) was observed in 3 duodenal GISTs with extracellular D-PAS(+) SF. **Ultrastructurally**,the 17 cases were classified as **myofibroblastic**(MFB), **GI autonomic nerve tumor-like**(GANT),**epithelioid**(EPL),and **ICC** types.The MFB type showed numerous dilated r-ER and solitary focal densities in intermediate filaments.The GANT type had neurite-like processes containing synapse-like structure and numerous neuroendocrine granules(NEG).The EPL type revealed abundant mitochondria,concentric membranous whorl bodies and many surface filopodia.The ICC type exhibited abundant intermediate filaments,rich s-ER and rare NEG.Ultrastructural SF were observed in 3 duodenal and 1 gastric GISTs.

Conclusions: Three duodenal GISTs showed extracellular skeinoid fibers,S-100 protein(+),and in 2/3 cases ultrastructural ICC type. **One gastric and 1 omental GISTs** showed prominent epithelioid and pleomorphic cells,CD34(-),SMA(+),higher MIB-1 index,ultrastructural MFB type,and **poor prognosis. Four ultrastructural types** were observed.The ICC type was only seen in the duodenal GISTs.**MFB** was mostly observed in those GISTs with plump spindle cells and epithelioid cells.Most of those cases were gastric GISTs and EGISTs. **GANT** was mostly seen in those GISTs with spindle cells. **EPL** was mostly observed in GISTs with epithelioid cells,including large,small round and pleomorphic cell morphology.MIB-1 index of GANT and EPL were **lower** suggesting better prognosis than MFB.

1584 Angiomatoid Fibrous Histiocytoma: Histopathologic and Ultrastructural Correlation

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Background: Angiomatoid fibrous histiocytoma (AFH) is a soft tissue neoplasm of uncertain differentiation which occurs most frequently in the extremities of children and young adults. Previous reports of ultrastructural features in AFH are varied and inconclusive.

Design: Electron microscopy (EM) was performed on 10 cases with histopathologic features of AFH. Tissue preserved in glutaraldehyde was available for 3 cases. Directed sampling of paraffin-embedded tissue for EM study was performed in the remaining cases with emphasis on areas of dense cellularity.

Results: The histopathologic features were consistent with AFH; the densely cellular regions were composed of round to spindle cells with eosinophilic cytoplasm and ill-defined cell borders. Nuclear pleomorphism was minimal. EM demonstrated the majority of cells to contain intermediate filaments that were rarely bundled. A dense external lamina surrounded the cells with focal intermingled collagen fibrils. A population of fibroblastic cells were identified with elongated and irregular nuclei, dilated RER and increased extracellular matrix. Histiocytic cells were also seen with abundant lysosomes. Scattered intracytoplasmic iron was present within lysosomes. Erythrocytes and serum were seen in intercellular sinusoidal spaces. Rare intracytoplasmic lumina were seen. Interspersed inflammatory cells including plasma cells and eosinophils were easily identified on EM.

Conclusions: Densely cellular regions directly sampled for EM demonstrated the most striking ultrastructural features; the neoplastic cells contained intermediate filaments and were surrounded by dense external lamina suggestive of pericyte-like differentiation. The presence of intermediate filaments is consistent with immunohistochemical staining for desmin reported in 50% of AFH cases. Cases which were not directly sampled demonstrated fibrohistiocytic features with increased collagenous matrix. Ultrastructural features of myofibroblastic cells were not prominent, as previously reported in ultrastructural AFH studies. Well-preserved ultrastructural detail obtained from paraffin-embedded tissue allowed for improved sampling of this heterogeneous lesion and support that AFH is a mesenchymal neoplasm with a tendency toward pericyte-like differentiation.

1585 Extraosseous Ewings Sarcoma of the Tongue Initially Mistaken for Lymphoblastic Lymphoma: Avoiding Diagnostic Pitfalls

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Background: Pediatric cancers are biologically and histologically distinct from tumors occurring in adults. While most adult tumors are of epithelial origin and defined by a specific organ system, pediatric malignancies tend to be embryonal in origin. Lack of morphologic differentiation in many of these tumors requires a multimodal diagnostic approach, especially when the tumor deviates from its classic presentation. This case report highlights key differences between adult and pediatric tumors, including appropriate triaging of tumor specimens and an integrated diagnostic approach.

Design: A 4 year-old female presented to her physician with difficulty swallowing. A polypoid mass at the base of the tongue was excised and the diagnosis of lymphoblastic lymphoma. Based on the diagnosis, the child underwent oncologic management. With failure of the residual tumor to respond, referral was made to a children's hospital with a request for pathology review. **Tissue for Study:** Formalin-fixed paraffin-embedded tissue was available for routine light microscopy, immunocytochemistry, electron microscopy (paraffin recovery) and RT-PCR.

Results: The tumor was composed of undifferentiated small round cells that had inconspicuous nucleoli and scant amphophilic cytoplasm. There was a fine capillary network, and the tumor cells appeared to be cohesive and closely apposed. Immunocytochemistry showed LCA and membranous CD99 reactivity, which occurs in lymphoblastic lymphoma/leukemia. CD3, CD20, desmin, myogenin, and NB-84 (neuroblastoma) were negative. Formalin-fixed paraffin-embedded tumor was recovered for electron microscopy. Ultrastructural examination identified primitive neuroectodermal features (rudimentary cell junctions, neurosecretory granules, neurite-like processes), suggesting extraosseous Ewings sarcoma. Based upon EM findings, tissue scrolls were taken from the paraffin blocks for RT-PCR. An EWS-FLI1 translocation [t(11;22)(q24;q12)] was discovered.

Conclusions: Ewings sarcoma may be identified by a combination of routine, immunocytochemical, electron microscopic and molecular techniques. A multimodal approach to pediatric small round cell tumors allows for the most appropriate diagnosis with difficult to distinguish childhood tumors that may occur in unusual sites.

1586 Giant Platelets and Absent Response to Ristocetin on Platelet Aggregation Assay: The Need for Electron Microscopy

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Background: Sebastian Syndrome (SS) and May-Hegglin Anomaly (MHA) are part of a spectrum of hereditary macrothrombocytopenias caused by a mutation in the MYH9 gene that encodes for the nonmuscle myosin heavy chain IIA. These disorders are characterized by thrombocytopenia with giant platelets, "Dohle-like body" leukocyte inclusions (DLBLI) with or without deafness, nephritis, or cataracts. Recent literature has suggested that the mutation of the MYH9 gene is also responsible for reduced platelet surface expression of glycoproteins (GP), specifically GPIb/IX/V. The case presented is of an infant and biological mother who have macrothrombocytopenia, DLBLIs, and no response to ristocetin on Platelet Aggregation Assays (PAAs).

Design: The patient is a 6-month-old male with a low platelet count since birth with no major bleeding or bruising episodes. The mother has a presumed history of ITP with giant platelets treated by splenectomy. There is no history of deafness, nephritis, or cataracts in the infant, mother, or other family members. Routine electron microscopy (EM) and whole mount EM with qualitative analysis were performed on the the mother's platelets and neutrophils and the infant's platelets. Platelet Aggregation studies were performed on the infant's blood (Chronolog Whole Blood Aggregometer).

Results: The mother's blood smear confirmed the presence of giant platelets and DLBLIs. EM showed the inclusions consisted of thin filaments and ribosomes that appeared to be arranged in an unorganized fashion. EM revealed the platelets to be markedly enlarged but with normal granule and dense body number and morphology. The infant's PAAs showed an absent response to ristocetin with an appropriate response to ADP, collagen, and arachidonic acid.

Conclusions: The MYH9 mutation is believed to be responsible for the enlarged platelets and DLBLIs in SS and MHA and EM is needed to differentiate between the two disorders. In the case presented, EM studies of the DLBLIs are suggestive of those seen in SS. The MYH9 mutation is also thought to cause a decrease in the platelet surface expression of GPIb/IX/V which would clinically result in a lack of response to ristocetin on PAAs. Decreased expression of GPIb/IX/V is seen in another hereditary macrothrombocytopenia, Bernard-Soulier Syndrome (BSS) as well as in Von Willebrand's Disease (VWD). BSS, while characterized by giant platelets, does not have DLBLIs. A concurrent VWD is possible and studies to evaluate for it are pending.

1587 Endolymphatic Sac Tumorigenesis; Ultrastructural Characteristics

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Background: While the endolymphatic sac has been well characterized by electron microscopy, VHL related tumors of the endolymphatic sac/duct remain without ultrastructural characterization.

Design: Our goal is to study and describe the electron microscopic findings of endolymphatic tumors (ELST) in these patients examining a spectrum of lesions from early small cystic structures to larger papillary lesions. Cases of ELST's from VHL patients were microdissected and processed for Transmission Electron Microscopy. Prior to ultrastructural analysis, VHL gene inactivation was demonstrated in tumor and cyst cells by demonstration of upregulation of HIF1, HIF2, and HIF targets CAIX and glut1.

Results: Consistently observed ultrastructural features in all tumors included basal lamina, desmosomes, microvesicles, microvilli, as well as intracytoplasmic accumulation of lipid. Occasionally or rarely observed were intracytoplasmic dense granules, abundant intracytoplasmic glycogen and mucin-like granules. Endolymphatic sac cysts (recently hypothesized to represent precursor lesions for ELST's) were characterized by flat epithelial cells with short microvilli, poorly formed desmosomes, and vesicles. More advanced papillary lesions consisted of multiple layers of columnar epithelial cells exhibiting desmosomes and junctional complexes as well as long surface microvilli and prominent lateral intercellular spaces.

Conclusions: Our data suggest that endolymphatic sac tumorigenesis is a multistep pathogenetic process, characterized by development of simple cysts into a complex epithelial neoplasm. The evolution of endolymphatic tumor formation is ultrastructurally characterized by acquisition of increasingly complex desmosomal contacts, glycogen, and mucin-like material.

1588 Something in Common for Lung and Endometrial Carcinoma

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Background: DC-LAMP was originally reported as a **L**ysosome-**A**ssociated **M**embrane **P**rotein expressed in mature dentritic cells. By comparing immunostaining spectrum of PE-10, (an anti-surfactant protein Ab), and DC-LAMP Ab in normal and neoplastic human lungs, using EM for cell definition, we have reported that DC-LAMP specifically stains Clara cells. We here explore specificity of DC-LAMP Ab and probable DC-LAMP ultrastructural localization.

Design: Composite epithelial and mesenchymal neoplastic tissue blocks were created including squamous and small cell lung carcinomas; endometrial and ovarian carcinoma; prostate adenocarcinoma; papillary thyroid carcinoma; colon and gastric adenocarcinoma; hepatocellular carcinoma; renal cell carcinoma; ductal breast carcinoma; schwannoma; alveolar soft part sarcoma; rhabdomyosarcoma; leiomyosarcoma; breast fibroadenoma; phylloides tumor; granular cell tumor and malignant melanoma. A tissue array of 26 endometrioid endometrial carcinomas (EndoCa), their normal endometrium, and simple and complex endometrial hyperplasias, was created. 7 EndoCa and normal endometrium were studied ultrastructurally.

Results: Only EndoCa stained for DC-LAMP, in an apical granular pattern resembling that of bronchioloalveolar carcinoma (BAC) (left figure panels). EM showed similar apical granules in BAC and EndoCa (right figure panels). Four of six endometrioid carcinomas had strong DC-LAMP expression in carcinoma and complex hyperplasia, but not in simple hyperplasia or normal endometrium. We are in the process of using immuno-EM to define the subcellular location of DC-LAMP.

Conclusions: Altered lysosomal trafficking and increased lysosomal proteases accompany tumor growth. Our data demonstrated that DC-LAMP is upregulated in secondary lysosomes in Clara cell type BAC and in endometrial carcinoma. Another Clara cell secreted 10-kDa protein (CC10) is reported to be also present in endometrium. CC10 was indicated as a regulator of malignant transformation in respiratory and urogenital epithelia. Our finding provides another evidence that a common regulator may be involved in the neoplastic growth of mucosal epithelial cells.