of the nodules aspirated was 1.8 (NNAN), 3.2 (HA), 3.0 (HCa) and 2.9 (PTC). The average numbers of nodules identified by US were 3.3 in NNAN, 2.0 in HA, 1.7 in HCa, and 1.8 in PTC ($p<0.05$). Furthermore, 40% (4 of 10) and 20% (2 of 10) of HCa were vascularized and microcalcified on US, respectively; and 50% (7 of 14) of NNAN had multiple (5) small nodules in the background thyroid. *FNA Findings* – the Hurthle cell tumors had more cellular smears, discohesive Hurthle cells, few, if any, lymphocytes, and scarce or absent colloid in comparison to the smears from NNAN.
Conclusions: Dominant thyroid nodules 2 cm or less on US without evidence of increased vascularity or microcalcifications in combination with the background thyroid containing multiple (3 or more) smaller nodules and the FNA smears containing some lymphoid aggregates with Hurthle cells in moderately sized sheets are likely to be benign. Communication between clinician and pathologist correlating US and FNA findings in difficult cases may avoid unnecessary surgery.

335 A 12 Year Analysis of 4,121 PAP Tests from 2,326 Adolescents of Southern Rhode Island: An Important Public Health Issue

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Background: Cervical cancer screening in adolescents is an important public health care issue because of the high risk of Human Papilloma Virus infection in teens. Although the American Cancer Society recently proposed a recommendation, there has been no consensus on when to initiate Pap smear screening, and how to manage teens with abnormal Pap smear due to the limited evidence-based long term studies.
Design: In this study, 4,121 Pap tests from 2,326 adolescents aged 12 to 19 at South County Hospital of Rhode Island from 1992 to 2004 were analyzed. The Bethesda system was used for classification of abnormal Pap smears. The majority of high-grade cases were confirmed by either biopsy or conization procedure. The prevalence of the abnormal Pap tests was compared to those of the general population over the same period from same database.
Results: The abnormal Pap tests in adolescents were significantly higher with 8.0 % atypical squamous cells of undetermined significance (ASCUS), 7.0% low-grade squamous intraepithelial lesions (LGSIL), and 2.2 % high-grade squamous intraepithelial lesions (HGSIL) in comparison with the general population (4.24 % ASCUS, 2.13 % LGSIL, and 0.48 % HGSIL). Of the 43 cases of biopsy confirmed HGSIL, there were seven cases of CIN 3 (carcinoma in situ) (16.3 %), four cases of CIN 2-3 (9.3 %) and 32 cases of CIN 2. Although about 80% of HGSIL occurred at ages 18 and 19, CIN 3 was seen in two 15-year-old teens. The average time between the first screening and detection of HGSIL was 14.8 months.
Conclusions: This study indicates that abnormal Pap smear in teens is a significant health care issue and that Pap smear screening should start early in sexually active teens or at 18 years of age if sexual history is not clear. Once initiated, teens should continue to have annual screen during adolescence regardless of number of previous normal screenings.

336 Suspicious for Malignancy in Fine Needle Aspiration of Breast: Reasons and Clinical Implications

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Background: Suspicious for malignancy (SFM) in fine needle aspiration (FNA) of breast is a diagnostic category that might cause dilemma in patient management. The diagnosis is rendered for a number of reasons, so its elimination may not be realistic. Its clinical implications need to be fully explored.
Design: To analyze the underline rationale for rendering the diagnosis and its clinical implications, 33 breast FNA specimens from 31 patients with the diagnosis of SFM were retrieved from our file in a 5-year's period from 2000 to 2004. 29 of the 31 patients had follow up histologic diagnosis, forming the basis of this study. The cytologic and histologic materials were reviewed and correlated.
Results: Of the 29 patients, follow up core biopsy, excisional biopsy and/or mastectomy revealed 20 invasive ductal carcinoma, 2 invasive lobular carcinoma, 3 ductal carcinoma in situ (DCIS), 2 atypical ductal hyperplasia, 1 ductal hyperplasia without atypia and adenosis, and 1 intraductal papillomatosis. Review of the cytologic materials identified the following factors that precluded more definitive classification of the lesions: 1. Scanty cellularity; 2. Predominantly cohesive cellular clusters; 3. Presence of myoepithelial cells; 4. Tumor cells in a cystic background with foamy cells; 5. Tumor cells with low nuclear grade; 6. Tumor cells with apocrine differentiation. Further analysis found that the most useful criteria for diagnosing carcinoma were hypercellularity, single cells, loosely cohesive clusters of cells with disorientation and irregular edge, significant anisonucleosis with high N/C ratio, prominent nucleoli and nuclear pleomorphism. When a combination of any four of the criteria was fulfilled, 14 of the 25 cases with carcinoma were correctly diagnosed. However, no reliable criteria were identified for definitive diagnosis of the carcinoma (including DCIS) with low nuclear grade and for recognizing atypical ductal hyperplasia. Therefore not all the cases could be accurately diagnosed cytologically with confidence. Excisional biopsy performed in 19 of the 29 patients provided with definitive diagnosis and the information necessary for proper patient management.
Conclusions: Multiple reasons are found for rendering the diagnosis of SFM in FNA of breast. Although possible to reduce the number of the cases by restrictively applying the identified criteria, it is unrealistic to eliminate the category. It is more important to set the appropriate way to manage the patients, and excisional biopsy is suggested as the next step.

Dermatopathology

337 CD10 and Ep-CAM Expression in Basal Cell Carcinoma, Classical Trichoepithelioma, and Desmoplastic Trichoepithelioma

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Background: The distinction between basal cell carcinoma (BCC) and trichoepithelioma (TE) has historically been made on the basis of specific histologic criteria, but it may be difficult when the tumor sample is limited. Recent reports have suggested a utility for CD10 and Ep-CAM immunostaining in recognizing BCC. Accordingly, this study was initiated in order to determine whether those markers might aid in the distinction between BCC and TE variants in small biopsy specimens.
Design: Paraffin sections of BCC variants, classical TE, and desmoplastic trichoepithelioma (DTE) were retrieved. Antibodies against CD10 (clone 56C6, Abcam, Inc) and Ep-CAM (clone Ber-EP4, Abcam, Inc) were applied using standard immunohistochemical technique. For CD10, the pattern of tumor cell staining was descriptively recorded as peripherolobular, solid, scattered, papillary mesenchymal body (PMB)-accentuated, or combined. Stromal labeling was similarly coded as continuous, discontinuous, or diffuse. Staining for Ep-CAM was categorized as 0 (negative); 1+ (1-20% of cells); 2+ (21-50%); or 3+ (≥51%).
Results: CD10 was present in at least a portion of tumor cells in 28 of 30 BCCs, 21 of 22 TEs, and 6 of 28 DTEs. The predominant pattern was peripherolobular in BCCs, PMB-accentuated in TE, and cord-like in DTE. Peritumoral stromal cells were labeled in all BCCs and TEs, and in 27 of 28 DTEs; the dominant pattern was diffuse-discontinuous in BCC, continuous in TE, and diffuse in DTE. Ep-CAM was present in all 40 BCCs, all 29 TEs, and 29 of 30 DTEs, but the mean percentage of immunoreactive tumor cells was 84%, 56%, and 31% respectively. The Ep-CAM staining pattern for both BCC and TE was peripherolobular. When positive, DTE again showed cord-like labeling for that marker. Analysis of immunostaining patterns in various morphologic BCC variants demonstrated no significant differences.
Conclusions: Although trends toward differential staining patterns for CD10 and Ep-CAM were evident in this analysis, those two determinants do not appear to provide practically useful information for the diagnostic separation of BCC from TE or DTE. Histologic criteria remain as the standard for recognition of those tumor types.

338 A High-Throughput Study Identifies Epithelial-Mesenchymal Transition as Major Determinant of the Melanoma Metastatic Capacity

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Background: Apart from Breslow index there are no histopathological or molecular markers that could consistently predict the metastatic potential for Cutaneous malignant melanoma (CMM) cases.
Design: To identify the genes associated with increased metastasis risk in a group of primary CCM with biological potential to metastasize (Vertical Growth Phase Melanomas cases with more than 1mm of Breslow's index), 36 primary CMM cases were analysed with the c-DNA microarray CNIO Oncochip ™ containing 6500 cancer-relevant genes. All patients had a minimum follow-up of 3 years in which 22 cases develop nodal metastatic disease and 14 not. The results were validated using immunohistochemistry in a Tissue Micro Array containing cores for an independent series of 132 primary CMM cases.
Results: Differences in expression between metastatic versus non-metastatic disease identified 116 differentially expressed genes at >2-fold ratio. Of these genes 91 were up-regulated and 25 were down-regulated. This set of genes included molecules involved in cell cycle regulation, epithelial-mesenchymal transition (EMT), angiogenesis, signal transduction, metabolism and structural genes. A large group of biologically significant genes was related with epithelial-mesenchymal transition (EMT). The validation in an independent series showed that proteins included in this group were associated with metastases development (p 0.002) and with a shorter survival time (p 0.009).
Conclusions: The study identified a specific subset of genes whose expression is related with the development of metastastic disease in patients with CMM. A significant proportion of these genes was related with EMT, the acquisition of a mesenchymal phenotype with migratory and invasive properties. This information could help to better understand the biology or this aggressive tumour and might result in new and more effective therapies or more accurate patient stratification.

339 Intracapsular Melanoma in Sentinel Lymph Node Biopsies

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Background: Sentinel lymph node biopsy (SLNB) is an important and significant independent factor for prognosis and management of patients with melanoma. Pathologically, sentinel lymph node (SLN) involvement by metastatic melanoma can be characterized, based on the location of metastatic foci of malignant cells, into subcapsular (most common), parenchymal, sinusoidal, or mixed. Potential pitfall in the interpretation of SLN for melanoma is the presence of benign nevic cells within the capsule (intracapsular) of the node. These characteristically lack cytonuclear atypia, mitotic activity and are usually immunonegative for HMB-45.
Design: Sentinel lymph node biopsies from two patients with proven cutaneous melanoma were processed using the SLNB protocol (modified from Cochran et al), and examined by routine light microscopy and immunohistochemical study (S-100 protein, Melan A, and HMB-45 in the first case, and Melanoma Cocktail in the second case)

Results: Intracapsular melanoma was identified in the two cases. In the first case, the melanoma cells were seen infiltrating and dissecting the capsular collagenous tissue (purely intracapsular), while the second case, the malignant melanocytes were found within the capsule as well as in subcapsular and parenchymal tissue. The capsular lesion was adjacent to a lymphatic vessel and had a smooth border. In both cases the melanocytic cells showed nuclear atypia and mitotic activity. The malignant melanocytes were immunopositive for HMB-45, and Melanoma Cocktail in the first and second cases, respectively.

Conclusions: We describe a new, but rare phenomenon of intracapsular pattern of metastatic melanoma in SLN, which could be a potential diagnostic pitfall in the microscopic interpretation. This could represent a new morphological step, that precedes the seeding of melanoma cells to other deeper regions of the lymph node.

340 Loss of Heterozygosity and X-Chromosome Inactivation Analysis of Primary and Epidermotropic Metastatic Melanoma

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Background: Loss of heterozygosity (LOH) has been previously demonstrated at multiple chromosome microsatellites in primary and metastatic melanomas. Epidermotropic metastases of melanoma are unique in their varied histomorphologic appearance which can mimic a primary lesion. Our objective was to compare LOH profiles in primary and epidermotropic metastatic melanoma (EMM) in order to delineate evidence for related clonality or independent origin of the metastatic lesions.

Design: We examined the pattern of allelic loss in the primary melanomas of nine patients in addition to twenty-one corresponding EMMs (average 2.3 metastases per patient). DNA samples were prepared from formalin-fixed, paraffin-embedded tissue sections using laser capture microdissection. Eight DNA microsatellite markers on six different chromosomes were analyzed: D1S214 (1p), D6S305 (6q), D9S171 (9p), D9S157 (9p), IFNA (9p), D10S212 (10q), D11S258 (11q), D18S70 (18q). In addition, X chromosome inactivation analysis was performed in tumors from females (N = 4).

Results: LOH was seen in 67% (6/9) of primary melanomas and 81% (17/21) of EMMs. The most frequent allelic losses in informative cases occurred at 10q (33%), 9p (22%), and 11q (22%) in primary melanomas, and at 10q (50%), 1p (44%), and 6q (39%) in EMMs. Primary lesions demonstrating LOH had concordant allelic loss in at least one locus in a corresponding EMM lesion in 83% (5/6) of cases. X-chromosome analysis showed non-random inactivation in 75% (3/4) and 71% (5/7) of primary melanoma and EMM cases respectively.

Conclusions: Our LOH and X-chromosome inactivation analysis data suggest that EMMs are clonally related to their primary lesion in many cases. Our data also indicated that some cases diagnosed as EMM may be divergent clones or, more likely, new primaries rather than metastatic disease. LOH and clonality assays could eventually be a useful tool in the confirmation of the diagnosis of EMM, and provide useful staging information.

341 Keratin 15 Stem Cell Marker and Other Immunohistochemical Characteristics of Sebaceous Neoplasms

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Background: Keratin 15 has been shown to represent a marker of multi-potent, adult epithelial stem cells in skin. An experimental transgenic mouse model, expressing a dominant-negative Lef-1 transcription factor, forms sebaceous tumors and has suggested that these tumors may be derived from the stem cell population in skin, and a single case report has shown CK15 staining in a sebaceous carcinoma. In order to evaluate for the presence and distribution of putative stem cells in the spectrum of human sebaceous tumors, "tumor stem cells," and to further characterize sebaceous neoplasms immunohistochemically we examined expression of the stem cell marker CK15, proliferation marker Ki-67, keratins 7 and CAM 5.2, and EMA in sebaceous carcinomas, adenomas and hyperplasias.

Design: Immunohistochemical analysis for CK15, Ki-67, CK7, CAM 5.2 and EMA was performed on formalin fixed, paraffin embedded archival tissue. Sections of 11 sebaceous carcinomas, 10 adenomas, and 7 hyperplasias were stained with monoclonal antibodies using standard immunohistochemical techniques. The tumors were evaluated for the presence and extent of staining, as well as for the pattern of staining within basal, suprabasal, differentiated sebocytes.

Results: In sebaceous carcinomas, CK15 showed scattered staining in basal and suprabasal layers. Adenomas showed more positivity in basal than suprabasal cells. In all three lesions, differentiated sebocytes exhibited the least reactivity with CK15. In carcinomas and adenomas the cells staining for Ki-67 were scattered thorough basal and suprabasal layer, with more cells at the basal layer. In hyperplasias Ki-67 reactivity was limited to the basal layer. Cytokeratin 7 staining was heterogenous, and CAM 5.2 was mostly negative. EMA showed diffuse positivity in most cases.

Conclusions: Sebaceous neoplasms are composed of a heterogeneous population of cells with various patterns of staining for the stem cell marker CK15, as well as other immunohistochemical stains. In carcinomas the putative stem cells are scattered, while in adenomas and in more differentiated carcinomas they are present mostly in the basal layer. The distribution of the stem cells and the proliferation marker Ki-67 seem to overlap, but more definite studies are needed. The results suggest that sebaceous tumors contain a subpopulation of "tumor stem cells" identified by keratin 15 staining.

342 A Panel of Immunoperoxidase Stains Useful in Highlighting the Intraepithelial Spread of Ocular Sebaceous Carcinoma and Differentiating It from Its Mimics

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Background: Sebaceous carcinoma is a rare, aggressive ocular malignancy that may exhibit intraepithelial pagetoid/bowenoid spread into the skin of the lid, the conjunctiva and even the cornea. The clinical and histopathologic diagnosis is difficult. The tumor is frequently misdiagnosed initially as in situ or invasive squamous cell carcinoma especially in small biopsy samples. A number of stains, including EMA and cytokeratin 8/18 (Cam 5.2) were previously used to diagnose sebaceous carcinoma. Because experience with these stains has not lead to a definitive diagnosis, especially in poorly differentiated cases, we propose evaluating these biopsies with a panel of more recently introduced stains to differentiate the intraepithelial spread of sebaceous carcinoma from other simulating lesions.

Design: 8 ocular sebaceous carcinoma specimens (3 orbital exenterations, 4 wedge biopsies and 1 biopsy) from 3 male and 5 female patients aged 46-93 were stained with a panel of antibodies including p16, p63, CK5/6, CK7 and Ki-67. The same panel of stains was applied to 6 in situ and invasive squamous cell carcinomas of the conjunctiva. We assessed the intensity, distribution and pattern of immunostaining of the tumor, the intraepithelial component, the surrounding lid epidermis and conjunctiva.

Results: Of the 8 sebaceous carcinoma cases 4 were moderately differentiated and 4 were poorly differentiated. All but one had intraepithelial spread into the lid and conjunctiva and one even to the cornea. Immunoperoxidase stains showed strong, diffuse positivity for p16 in 7/8 cases (both tumor and intraepithelial spread), while the epidermis and conjunctiva were negative.

Immunohistochemical staining pattern of ocular sebaceous carcinoma and squamous cell carcinoma.

	CK5/6	p16	p63	Ki67	CK7
Sebaceous Carcinoma	0 (8/8)	3+ (7/8)	3+ (6/8)	3+ (2/8)	3+ (6/8)
Intraepithelial and Invasive Squamous Cell Carcinoma	3+ (6/6)	0 (6/6)	3+ (6/6)	3+ (5/6)	0 (6/6)
In Situ Conjunctiva					
Normal Conjunctiva	3+ (14/14)	+/- (14/14)	1+ (14/14)	0 (14/14)	1-2+ (14/14)
Normal Lid Epidermis	3+ (14/14)	0 (14/14)	1+ (14/14)	+/- (14/14)	0 (14/14)

Conclusions: A panel of immunostains including p16, p63 and Ki-67 is useful in identifying the intraepithelial component of ocular sebaceous carcinomas while stains for CK5/6 and CK7 can help differentiate between sebaceous carcinoma, usually CK7(+)/CK5/6(-) and squamous cell carcinoma, commonly CK7(-)/CK5/6(+).

343 Adnexal Clear Cell Carcinoma with Comedonecrosis: Clinicopathological Analysis of 12 Cases

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Background: *'Clear cell changes'* are a prominent feature in a wide variety of squamous and adnexal carcinomas. Pure clear cell carcinomas of the skin are exceptionally rare.

Design: We report a series of 12 cases of a distinctive cutaneous adnexal carcinoma showing squamous differentiation, comedo-necrosis and prominent clear cell change, capable of an aggressive clinical course.

Results: Adnexal clear cell carcinoma with comedonecrosis (ACCCC) is a tumor of older individuals (median age=71 years) and occurs with the same frequency in both sexes. It has predilection for the head and neck region and is most common on the scalp, but there is a wide anatomical distribution including extremities and genital areas. Three patients were transplant recipients. Clinically, the lesions are erythematous to flesh colored, solitary papules, nodules or tumors, frequently ulcerated with surface scale crust. Size of the tumor can vary from less the 1 cm to several centimeters in diameter. Rapid growth of the lesion is commonly reported. Histologically, most lesions were initially considered as a variant of squamous cell carcinoma or tricholemmal carcinoma. They showed nested, multilobular or trabecular epithelial tumor infiltrating the dermis with a poorly marginated advancing border. At low power, most lesions appeared squamoid with occasional formation of squamous pearls. Individual tumor nests showed a distinctive zonal arrangement with outer squamoid cells merging with centrally located clear cell areas containing foci of comedonecrosis. Nuclear pleomorphism varied from case to case and was at least moderate in most cases. The mitotic count ranged from 2 to 32 per mm^2 (median 8 per mm^2). None of the lesions showed evidence of ductal, cuticular or apocrine differentiation. Immunohistochemical profile of ACCCC include expression of epithelial membrane antigen (EMA) and cytokeratin 17 and focal staining with CEA in some cases. ACCCC appears to be a potentially aggressive neoplasm as 4 cases recurred locally and 2 cases had regional and distant metastases within the average follow-up period of 37 months.

Conclusions: ACCCC appears to be a distinctive adnexal neoplasm which has to be distinguished from more indolent squamous and tricholemmal carcinomas.

344 Primary Cuteneous Adenoid Cystic Carcinoma: A Clinicopathologic and Immunohistochemical Study of 17 Cases

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Background: Primary adenoid cystic carcinoma of the skin is a rare neoplasm with only one large series in the English literature.

Design: Our study of 17 cases, from the authors' referral archives (PR, EC), aims to help further characterise this entity, as distinct from its salivary gland counterpart.

Results: Clinically, the lesions presented as solitary slow growing nodules (duration 6 months to 4 years), with a male predilection (n=11). The age distribution ranged from 24 to 81 years, however, most commonly occurred in middle-aged to elderly individuals

(median=70 years). Lesions were located on the scalp (n=5), extremities (n=4), face (n=3), trunk (n=2), flexural sites (n=2) and abdomen (n=1). Surgical excision was the treatment of choice. Histologically, all lesions were composed of variably-sized dermal aggregates of basaloid cells with at least focal cribriform pattern, disposed in a fibrous stroma. In 9 cases solid or tubular arrangements predominated. Many cell clusters contained hyaline intercellular basement membrane material highlighted by collagen type IV and laminin. The tumour cells, in all cases, were small to medium-sized with small nucleoli and scanty amphophilic cytoplasm. Perineural invasion was confirmed in 12 cases. Mitotic figures were rare (Median=1.5/1mm^2). All patients showed infiltration into deep dermis with involvement of subcutaneous fat (n=17). The tumour cells demonstrated expression for cytokeratin (11/11), epithelial membrane antigen (14/14) and carcinoembrionic antigen (glandular lumena –14/14). The outer myoepthelial layer was highlighted by smooth muscle actin (7/12). Focal S100 protein expression was also noted (9/12). Clinical follow-up (range: 1-25 years, median=3 years, n=14) showed 2 locally recurrences (12%), one of which presented 4 years after the original diagnosis and excision. To date, no metastases have been recorded.

Conclusions: Our study supports findings of previous studies that primary cutaneous adenoid cystic carcinoma is a distinct entity, with a more indolent behaviour than its salivary gland counterpart. The lesion may recur locally and has a low grade malignant potential.We hope this study of 17 cases helps in the characterisation of this extremely uncommon tumour.

345 Cytokeratin 18 in Melanoma Cells: Assessment with Immunohistochemistry, Western Blot Analysis, In Situ Hybridization, and Reverse Transcription Polymerase Chain Reaction

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Background: It has been generally accepted that malignant melanoma (MM) lacks cytokeratin (CK) expression and this serves to differentiate MM from poorly differentiated carcinomas. A few reports indicated the presence of CKs (including CK18) in some types of melanoma or cultured melanoma cells but this has not been fully explored. In this study, we systematically investigated the presence of CK18 in four well characterized melanoma cell lines by multiple protein and mRNA assays.

Design: MM cell lines A375, A875, M14, and SK-Mel-1 were used, and phenotypically verified by immunostaining with S-100, HMB45, and MART-1. The expression status of CK18 was investigated by immunocytochemistry of cell blocks, and Western blot analysis of total protein extracts, using the monoclonal antibody DC-10 (Zymed). The mRNA expression status of CK18 was analyzed by in situ hybridization with a digoxigenin-labeled cDNA probe and by reverse transcription polymerase chain reaction (RT-PCR) with intron-spanning primers designed for specific detection of CK18 mRNA (upstream, 5'-AGATCATCGAGGACCTGAGGGCTC-3', downstream, 5'-ATGTCGTTCTCCACAGACTGGCG-3'). In addition, archived primary cutaneous and mucosal melanoma tissues from 49 cases were studied for reactivity with CK18 antibody by immunohistochemistry. Positive and negative controls were employed to rule out false positivity.

Results: The protein and mRNA of CK18 were detected in the four melanoma cell lines by immunocytochemistry, Western blot analysis, in situ hybridization, and RT-PCR, with A875 having the highest level. CK18 positive melanoma cells were observed in 18% (9/49) of MM tissue samples, but the staining was only focal or scattered in distribution, and weak to moderate in intensity, as compared to positive controls of epithelial neoplasms.

Conclusions: CK18 appeared to be constitutively present, in variable amounts, in the four melanoma cell lines studied. Although positivity of CK18 in melanoma tissues differed from melanoma cell lines and was limited and weaker than epithelial neoplasms that were known to express CK18, these data prompt caution in applying and interpreting CK18 protein or mRNA assays of either tissue or cytological samples, and in analyzing cultured cell lines.

346 Expression of Dicarbonyl/L-Xylulose Reductase (DCXR), a Novel Cell Adhesion Molecule, in Melanocytic Lesions

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Background: Using the laser capture microdissection-micro serial analysis of gene expression (LCM-micro SAGE) (Mod Pathol. 2005,18:577), we previously identified dicarbonyl/L-xylulose reductase (DCXR) as a marker for prostate cancer. Virtual Northern blot of 189 SAGE libraries also revealed high *dxcr* expression in malignant melanoma. Recently, we have found that DCXR colocalizes with β-catenin and E-cadherin in prostate epithelial cells suggesting that this protein may function as a cell adhesion molecule. As several studies have demonstrated abnormal expression of adhesion molecules in melanoma progression, we investigated the expression of DCXR in a spectrum of melanocytic lesions.

Design: Expression of DCXR was assessed in a tissue microarray with 53 benign and malignant melanocytic lesions: 10 benign nevi (BN), 10 dysplastic nevi (DN), 14 primary melanomas (MM), and 19 metastatic melanomas (MMM). Immunohistochemical analysis was performed using a polyclonal anti-DCXR antibody. The percentage of positive cells, intensity and subcellular distribution were scored for each case. Differences were analyzed by Fisher's-exact test.

Results: BN, DN, and MM strongly expressed DCXR in the intraepidermal component with both cytoplasmic and membranous patterns. Overall the intensity of expression was stronger in BN and DN compared to MM and MMM ($p<0.005$). Nevus cells in the dermal, "maturing" component in BN and DN expressed less DCXR with a predominantly cytoplasmic pattern. Most (17/19) MMM (89.5%) expressed DCXR. However, only 7 (41%) of the positive cases showed a membranous pattern. Interestingly, perinuclear (Golgi-like) expression of DCXR was found in melanoma cells with a dishesive pattern of growth (particulary in MMM). The nuclear localization of DCXR was more predominant in BN and DN than MM and MMM (20 vs 5%). Regarding epithelial cells, DCXR was strongly expressed on the intercellular bridges of normal squamous epithelium, suggesting a participation of this molecule in adhesion between these cells.

Conclusions: The phenotypic change from membranous to cytoplasmic localization and the decreased expression of DCXR in mature, dermal nevus cells is similar to that seen with other markers such as gp100. The weaker expression of DCXR in melanoma cells may represent a higher invasive potential in melanoma cells. This possibility is further supported by the observed cytoplasmic (or Golgi) rather than membranous pattern of expression in dishesive melanoma cells.

347 Clinicopathologic and Immunohistochemical Features of Aggressive Cutaneous Squamous Cell Carcinomas

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Background: Cutaneous squamous cell carcinomas (CSCCs) generally show relatively benign behavior. However, in a minority of cases these lesions may behave aggressively assuming a very malignant course, especially in patients who are immuno-compromised. These tumors tend to rapidly develop into regionally destructive and recurrent lesions. There is a paucity of data regarding the clinical and immunohistomorphologic features of these aggressive tumors.

Design: Patients with aggressive CSCCs (tumors with rapid growth and large size, >1cm) were identified from the clinicopathologic archives. Any history of altered immunity was noted. Clinicopathologic features (ie. tumor size, histologic grade, depth of invasion, and subtypes) and immunohistochemical (qualitative and quantitative) profiles were studied using tissue microarray and Ariol imaging system on formalin fixed paraffin embedded tissue. Statistical analysis was performed.

Results: We identified 33 patients who had unusually large CSCCs (average 3.9 cm, range 2.0 to 9.5 cm) with rapid growth (within months). All were caucasian males ranging in age between 48 and 92 years (average 62 years). Nearly all patients had a significant clinical history of altered immunity: History of chronic leukemia (n=6), history of visceral malignancy (n=3), history of renal transplantation (n=2), chronic renal disease (n=6), DM (n=8), HIV (n=1), immunosuppressive treatment (n=5). Tumor size: 2.0 cm - 9.5 cm; average size 3.9 cm. Tumor depth: <2mm (n=6), 2-5 mm (n=17), >5 mm (n=10). Differentiation: 11 poor, 16 moderate, 6 well differentiated. Tumor subtypes: acantholytic (n=1), verrucous (n=3), desmoplastic (n=6). 27 tumors invaded into deep dermis while 6 were in the subcutis. Two cases had regional lymph node metastases and 12 showed local recurrence. Vascular (n=6) and perineural (n=11) invasion was seen. Average mitotic activity was >2/10 hpf. Immunohistochemistry showed decreased expression of E-cadherin (29/33), and overexpression of cyclin-D1 (16/16) and p53 (33/33). The average Ki-67 proliferative index was 80%.

Conclusions: 1. Aggressive CSCC, as defined by rapid growth and large size, occur more commonly in immunosuppressed individuals. 2. Microstaging of CSCC employing histologic grade, level of invasion, and tumor thickness, may offer a more accurate assessement of the likelihood of both recurrence and regional spread. 3. Aggressive CSCCs typically have a high proliferative rate and show overexpression of cyclin-D1 and p53 with decreased expression of E-Cadherin.

348 Can p16 Expression Help Distinction of Spitz Nevus from Melanoma?

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Background: Mutations in the gene CDKN2A, which encodes for p16 and p14ARF, have been demonstrated in familial melanoma. 50% of familial melanomas and 25% of sporadic melanomas are associated with loss of p16. p16 acts as a tumor suppresser by inhibiting CDKI (cyclin-dependent kinase inhibitor) which in turn renders the retinoblastoma protein inactive resulting in cell-cycle arrest. As loss of p16 is frequent in melanomas, we explored the possibility of using its expression to differentiate Spitz nevi from malignant melanomas.

Design: 58 skin biopsies with common benign nevus, Spitz nevus, melanocytic nevi with architectural disorder (Clark's nevi), invasive melanoma and metastatic melanoma were randomly selected, without knowledge of hereditary mutations in CCKN2A. Cases with co-existing melanoma and benign nevus were excluded. 4 micron sections were cut and stained with p16 using standard immunohistochemical protocol and pre-digestion with proteinase K. Cytoplasmic or nuclear expression in lesional cells was considered positive. p16 expression was analyzed by two pathologists at high power (to avoid bias on architectural basis), and without knowledge of the prior diagnosis.

Results: The cases included- 16 cases of common benign nevi (M:F =4:12; age 25 – 48 years, mean 36 years), 11 Spitz nevi (M:F =6:5; age 1-37 years, mean 10.5 years), 9 nevi with architectural disorder (M:F= 2:7, age 9 – 60 years, mean 36 years), 15 melanomas (M:F = 6:9; age 19 – 76 years, mean 50 years), and 7 metastatic melanomas (M:F =5:2, age 47-83 years, mean 63 years). The results for p16 expression are shown in table 1.

Conclusions: 1. p16 expression was maintained in 88% of benign common melanocytic nevi and 36% of Spitz nevi. 2. p16 expression was lost in all primary and metastatic melanomas. 3. p16 expression pattern in nevi with architectural disorder was not distinctly different from melanomas. 4. p16 expression analysis can be of value in differentiating Spitz nevi from malignant melanoma.

Table1

Type of Melanocytic Neoplasm	# cases	positive	negative	% positive	% negative
Benign common melanocytic nevi	16	14	2	88	22
Spitz nevi	11	4	7	36	64
Melanoma	15	0	15	0	100
Metastatic melanoma	7	0	7	0	100
Nevus with architectural disorder	9	2	7	22	78

349 Leukemia Cutis: A Histologic, Immunohistochemical and Clinical Review

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Background: Leukemia cutis (LC) is an uncommon condition that may cause diagnostic difficulty. LC occurs in the setting of preexisting leukemia, or can present as the initial finding with no evidence of systemic disease (aleukemic LC). We undertook a clinicopathologic review of 23 cases of LC.

Design: 23 cases of LC were identified. Information on clinical parameters and primary underlying hematologic disease was retrieved. Available slides were reviewed. In 16 cases blocks were available for immunohistochemical stains (CD23, CD34, CD56, CD68, CD117, lysozyme, MPO and CD4). Follow up was obtained.

Results: Ages ranged from 10 mo to 88 yrs (mean, 53 yrs), with 14M:9F. Hematologic diagnoses included acute myeloid leukemia (AML) (n=14), myelodysplastic syndrome (MDS) (n=3), T-cell acute lymphoblastic leukemia (T-ALL) (n=1), B-cell acute lymphoblastic lymphoma (B-ALL) (n=1), essential thrombocythemia (ET) (n=1), and leukemia, NOS (n=3). In 15 patients, the diagnosis of LC followed the diagnosis of the underlying disease. The interval from primary hematologic diagnosis to presentation of LC ranged from 0 to 24 months (mean, 6.6 months). In 4 patients with AML and 1 patient with B-ALL, LC occurred concomitantly with leukemic transformation. The underlying diagnosis established in 3 cases of aleukemic LC included ET, MDS and AML. The WBC counts in 19 patients ranged from 1,100 to 692,000 (mean, 107,715). There was no statistical difference in WBC in patients presenting with LC and concurrent systemic disease versus when systemic disease preceded (p= 0.162). LC presented as erythematous lesions, purple nodules/plaques, and maculo-papular eruptions. LC eruption was usually multifocal without site predilection. LC was characterized by nodular and diffuse infiltrates within the dermis and often subcutis. In 5 cases, incomplete granulocytic maturation and dyspoiesis was evident. LC resulting from AML M4 and 5 had 100% positivity with CD68 and lysozyme. Follow-up, available on 7 cases (mean, 5 years), showed 5 DOD, 1 AWD, and 1 ANED. The underlying diagnosis for the 5 DOD included, AML M5 (n=1), AML M4 (n=1), AML M3 (n=2) and MDS (n=1). The interval to death, after diagnosis of LC ranged from less than 1 week to 2 years (mean 1 year).

Conclusions: - LC may precede the diagnosis of systemic disease in greater than 10% of cases. - LC is most frequently observed in the setting of AML, especially in AML with monocytic differentiation. - Lysozyme and CD68 are the most useful stains for identifying LC with monocytic differentiation. They should be used in the setting of aleukemic LC.

350 Pathology-Driven Immediate Lymphadenectomy for Primary Melanoma: Impact on Staging, Prognostication and Outcome

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Background: Best management of early melanoma remains controversial, especially management of regional nodes. An international randomized trial tested the capacity of sentinel node biopsy to identify who may benefit from immediate lymphadenectomy.

Design: 2001 Primary melanoma patients were randomly assigned to wide excision/observation, with lymphadenectomy if nodal recurrence developed; or wide excision/sentinel node biopsy, with immediate lymphadenectomy if the biopsy contained metastases of melanoma.

Results: In 1327 primary aim patients with intermediate-thickness primary melanoma (Breslow 1.2-3.5 mm), disease-free survival at 5 years median follow-up was significantly higher in the nodal biopsy group than after nodal observation (78.1% vs. 72.6%; hazard ratio, 0.74; 95% confidence interval, 0.59 to 0.92; P= 0.008). At this interim analysis (third of five planned) overall melanoma-specific survival was not different. Five-year survival was significantly higher when patients with sentinel nodal metastases (detected by HE or immunohistochemistry) were managed by immediate (71.2%) rather than delayed (53.4%) lymphadenectomy (hazard ratio, 0.52; 95% confidence interval, 0.33 to 0.81; P=0.004). The frequency of sentinel nodal metastases found at biopsy (15.9%) matched the incidence of nodal disease that develops during nodal observation after wide excision of the primary (15.9%). The observed group had more tumor-involved nodes when they eventually came to surgery (3.6 vs. 1.4; P<0.0001), indicating that their disease progressed substantially during the period of observation. The presence or absence of tumor in the sentinel node was the most sensitive and accurate predictor of clinical outcome: 5-year survival was 89.9% for patients with tumor negative sentinel nodes and 71.3% for patients with tumor in the sentinel nodes (hazard ratio, 2.51; 95% confidence interval, 1.60 to 3.96; P<0.001).

Conclusions: Patients with primary melanomas (1.2-3.5 mm) should be staged by sentinel node biopsy and the need for additional surgery determined from the pathology of the sentinel node. The role of the pathologist is thus critical in these procedures. Sentinel nodes need to be sectioned at multiple levels and examined by HE and immunohistochemical staining. In the future it is likely that the amount and disposition of tumor in the sentinel node will determine the need for completion lymph node dissection, a procedure that carries substantial morbidity.

351 Comparison of UVB Induced Gene Expression Profiles in Whole Skin from Melanoma Patients and Healthy Controls

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Background: Ultraviolet (UV) irradiation increases melanoma risk by poorly understood molecular events. Recent studies have used DNA microarrays to study transcriptional responses to UVB irradiation in cultures of pure keratinocytes or melanocytes alone. To better understand whether such changes are relevant in the context of the epidermal melanin unit (EMU) *in vivo*, we have examined gene expression profiles of human whole skin before and after UVB irradiation.

Design: Under an IRB approved protocol, pairs of 8 mm skin punch biopsies from non-sun exposed skin were obtained from three patients with a history of melanoma and three control patients. One punch of each specimen pair was either UVB irradiated *ex vivo* (total dose 80 mJ/cm^2) or sham irradiated prior to RNA harvest. Isolated RNA was used for biotinylated target synthesis and hybridization to Affymetrix Human U133Av2 GeneChip gene expression microarrays. After normalization and filtering, microarray expression data was examined by the Significance Analysis of Microarrays algorithm to identify candidate gene transcripts whose expression was consistently altered in UVB irradiated skin compared to sham treated skin in either melanoma patients and/or healthy controls.

Results: Several transcripts were identified that demonstrated differential UVB-induced expression changes in melanoma patient skin versus healthy controls. For example, expression of retinoblastoma binding protein 6 increased an average of 1.4 fold in melanoma patients after UVB exposure, but not in healthy controls. Due to small sample size, however, these changes did not reach statistical significance. When melanoma patients and controls were considered as a single group, we identified 47 genes that demonstrated statistically significant gene expression changes after UVB irradiation. Among these were Rab38 and Rab31 (each averaging 0.8 fold change), two genes of the MAPK signaling pathway that is constitutively activated in the early stages of melanoma development.

Conclusions: We have identified several gene transcripts whose expression appears to be uniquely altered in UVB irradiated skin. To investigate if the changes identified in this model are relevant to human melanoma progression, we plan to use laser capture microdissection and quantitative RT-PCR to confirm differential expression in primary human melanoma samples and to identify the specific cell populations within the EMU responsible for the observed UVB alteration patterns.

352 Pyogenic Granuloma-Like Polypoid Pseudolymphoma of the Skin: 12 Cases of a Distinct New Entity

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Background: A distinctive new entity of polypoid inflammatory skin lesions typically presenting as a solitary erythematous raised papule in middle aged to elderly patients and favoring the head and trunk is described. It is dissimilar to cases documented in young children and adolescent presenting as multiple papules and nodules in acral sites first described in 1988 under the rubric 'acral pseudolymphomatous angiokeratoma of children' (APACHE).

Design: Paraffin blocks and slides of all cases were retrieved from the routine and consultation case files of one of the authors (E.C.). The routine histology and appropriate immunohistochemistry (IHC) were performed. PCR-based analysis of B and T cell clonality was undertaken where possible. Available clinical follow-up information was obtained.

Results: The lesions occurred typically in middle-aged adults (mean age 38.5 years) with a slight female predominance (9/12 cases). They presented mostly as a solitary erythematous raised papule, ranging in size from 2.5 to 9 mm (mean 5.54 mm), located mainly on the head and trunk. Only one case presented as multiple papules. The preferred clinical diagnosis was pyogenic granuloma in most cases. Cases with follow up show no recurrence in excised lesions. Histologically, all cases were well-circumscribed polypoid exophytic lesions bordered by an epithelial collarette and uniformly characterized by a dense dermal inflammatory infiltrate of lymphocytes with minimal cytologic atypia and admixed variable numbers of plasma cells and histiocytes. Scattered eosinophils were identified (7/12). Small germinal centers were identified in one lesion. There were prominent vascular channels lined by plump endothelial cells, the cytological appearances reminiscent of high endothelial venules of lymph nodes. Immunohistochemistry demonstrated a predominance of CD3+ T cells with a CD4+ immunophenotype and a minor population of CD20+ B cells. Kappa and lambda light chain IHC and PCR studies showed no evidence of monoclonality.

Conclusions: The current series is the first and largest so far in the characterization of this distinctive new entity. The reactive nature of these lesions is supported by the lack of clinical recurrence and the absence of monoclonality of the lymphoid cells. We propose this unique lesion to be denominated as 'pyogenic granuloma-like polypoid pseudolymphoma' of the skin.

353 Characterization of Inflammatory Cells in Fixed Drug Reaction, Erythema Multiforme, Stevens-Johnson Syndrome and Toxic Epidermal Necrolysis Spectrum of Interface Dermatidities

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Background: Cutaneous hypersensitivity reactions of the interface type manifest as a spectrum of disorders from localized reactions known as fixed drug reaction (FD), to more systematized reactions limited to the skin known as erythema multiforme (EM), to reactions with mucosal involvement known as Stevens-Johnson syndrome (SJS) and lastly to reactions with skin sloughing and significant mortality known as toxic epidermal necrolysis (TEN). Skin biopsies of these disorders can appear identical by light microscopy. However, differentiation is clinically important to predict the ultimate severity of disease in affected individuals. The rarity of the severe drug reactions, namely SJS and TEN, makes studying these entities difficult. The aim of this study is to analyze the phenotype of cells participating in these reactions to determine if there are differences using immunophenotypic parameters.

Design: 5 micron sections of biopsies from 18 FD, 17 EM, 5 SJS and 5 TEN were stained for CD-4, CD-8 and CD-68 using a Dako Envision automated stainer. Cells of each phenotype were counted and averaged over 3 high power fields in each of the epidermis, epidermal-dermal interface and upper dermis. The number of each cell type in each micro-anatomic compartment was compared across diagnostic groups. CD4:8 ratios between less severe cases (FD and EM) and more severe cases (SJS and TEN) were compared by Mann-Whitney test.

Results: All of these disorders had a predominantly CD8 positive infiltrate in all compartments. No difference was found among the analyzed groups for CD4 within the epidermis and interface compartments. The dermal CD4 and CD8 infiltrates are shown in the table below.

	Mean Dermal CD4 & CD8 Lymphocytes		
	CD-4	CD-8	CD4:8
FD	8.9	31.0	0.32
EM	7.9	28.5	0.29
SJS	2.7	17.8	0.15
TEN	0.7	20	0.06

There is a decreasing trend in CD4 cells with higher grade lesions as well as in the CD4:8 ratio (p=0.09). CD68 did not differ among the groups.

Conclusions: There is a progressive reduction in CD4:8 from FD to EM to SJS to TEN in the dermal compartment. This reduction is primarily a function of reduced CD4 lymphocytes in the dermis. The trend for more severe lesions to have fewer CD4 cells may be important in the underlying immunologic nature of these severe idiosyncratic drug eruptions. This observation could be useful in the analysis of biopsies with clinical concern for SJS-TEN progression; however greater numbers are needed for clinical application.

354 Molecular Pathology of Stage I and II Cutaneous Malignant Melanoma: Study of the Sentinel Lymph Node

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Background: Lymph node mapping and sentinel node biopsy are currently used to stage patients with cutaneous malignant melanoma (MM). Immunohistochemical stains, used in combination with the routine histological examination of the sentinel nodes (SN), increase the ability to detect micrometastasis; however, molecular biology techniques (MBT) may improve the diagnostic sensitivity.

Design: Forty five SN, negative on routine histology, from 598 cases of MM were included in this study. A central slice of the SN was cryopreserved and the remainder of the tissue was formalin fixed and paraffin embedded. The primary lesions were MM stage I or II, with a follow-up larger than 2 years. The SNs were studied with hematoxylin-eosin (HE), immunohistochemistry (IHC) for S-100 and HMB-45, and MBT (RT-PCR) for the detection of tyrosinase cDNA.

Results: In 10 of the cases (22.2%), tyrosinase was detected by RT-PCR; 3 of these cases were also positive by IHC. The population was divided into three groups: I - HE-/IHC+/MBT+ (3 cases); II - HE-/IHC-/MBT+ (7 cases); III - HE-/IHC-/MBT- (35 cases). Correlation of the groups with survival showed: I - 2/3 died (67%); II - 4/7 died (57%) and III - 35/35 (100%) of the patients are alive, with no lymphadenectomy and a median follow-up of 60 months.

Conclusions: The addition of MBT appears to be of great value for the detection of SN micrometastases in subjects with cutaneous malignant melanoma. In our series, those subjects who showed negativity with all three methods had a null recurrence rate. Therefore, this triple negativity could be a positive prognostic factor for overall survival. Our findings suggest the possibility of a molecular oncological staging, which would allow the selection of patients with submicroscopic metastases for a complete treatment.

355 LYVE-1 and D2-40, Two Lymphatic Specific Markers, Increase Detection of Lymphatic Invasion in Cutaneous Melanoma

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Background: Our previous results showed that the extent of peritumoral lymphangiogenesis, growth of new lymphatic vessels, can predict metastasis to sentinel (Dadras et al, 2005) and distant lymph nodes (Dadras et al, 2003), as detected by lymphatic endothelial hyaluronan receptor-1 (LYVE-1)-positive peritumoral vessels. We recently identified podoplanin, a mucin glycoprotein, as the antigen recognized by D2-40 antibody, which is commercially available (Schacht et al, 2005). Lymphatic vascular invasion by malignant tumor cells may increase the risk of relapse and/or decrease disease-specific survival in patients with malignant melanoma. Since the detection of lymphatic vascular invasion by tumor cells, using routine hemotoxylin and eosin-stained (H&E) sections, is equivocal, we used anti-LYVE-1 and anti-podoplanin (D2-40) antibodies to enhance detection rate.

Design: Of a total of 83 patients with primary malignant melanoma, 38 patients had late stage melanoma metastasis to distant lymph nodes or were disease-free (mean follow up=6.5 years). The remaining 45 patients had sentinel lymph node biopsy; 18 were positive and 27 were negative for melanoma metastasis to sentinel lymph nodes. Routine histological evaluation of tumor H&E sections did not show definitive vascular invasion. We immunostained at least two primary tumor sections for LYVE-1 (a generous gift from D.G. Jackson; 1:600) and D2-40 (Signet; 1:100 dilution).

Results: At least 11% of melanomas showed unequivocal lymphatic invasion, defined as LYVE-1-positive or D2-40-positive vessels containing melanoma cells within the lumen. This finding was localized to the periphery of the tumor showing foci of dermal invasion and significantly correlated with lymph node metastasis (P = 0.04).

Conclusions: Our results show, for the first time, lymphatic specific markers can reliably identify lymphatic vascular invasion, which is significantly correlated with distant or sentinel lymph node metastasis of cutaneous melanoma.

356 Cutaneous (D) Primative Neuroectodermal Tumor/Ewing Sarcoma (PE): Same Tumor as Deep PE or New Entity? 15 Cases

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Background: PE are generally large, in deep soft tissue or bone of young patients with male predominance, round cell morphology, vague rosetting, PAS positive, CD99 membrane staining, t(11;22) positive, and poor behavior. Reported DPE (1-5 cases each) from 1-2 decades ago: little or varying molecular data, notably better behavior. We wanted to review our DPE.

Design: Cases coded as "skin PE" were analyzed. Only intradermal cases (+/-subcutis involvement) were included. Excluded: insufficient material, skeletal muscle or bone involvement, or otherwise diagnosed.

Results: 15 cases:11 F and 4 M. Ages ranged 2-76, median 28, mean 33 years. Locations: thigh/groin (5), back/shoulder (3), neck (2), and 1 each: chest, scalp, forehead, hand, foot. All cases: clinically considered benign (cyst, lipoma, bug bite, vascular tumor), painful, and present for weeks-1 year. Tumor sizes ranged 0.5-2.3, median 1.5, mean 1.3 cm. Original diagnosis ranged from benign adnexal tumor to lymphoma and Merkel cell carcinoma; only one considered DPE. All cases were deep demal, only one of papillary dermis (pedunculated); 9 also subcutis. All: circumscribed (except metastasizing case) with pseudocapsule, vague rosettes, round smooth-contoured tumor nuclei with fine chromatin, indistinct nucleoli, scant pink to clear cytoplasm and collagneous stroma. Mitoses (median 8/10), necrosis (3). IHC: All CD99 membrane, vimentin, PAS, 2 focal synaptophysin, 1 focal NSE positive, 1/1Fli-1+. All negative: chromogranin, NFP, keratins, CK20, EMA, CEA, desmin, actin, S100, HMB45, LCA, CD20, CD79a, tdt, CD3. t(11;22): positive (1), negative (4), inability to amplify (4). Treatment: wide excision, chemotherapy (9) and radiation (6). Follow-up (13): 1 metastasis to stomach (DOD, 2 years) but 12 with no evidence for recurrence/metastasis,1.7-10; mean 7.3, median 8 years.

Conclusions: DPE, primarily small circumscribed tumors of adult female thigh, deep dermis +/- subcutis, has good behavior but should be distinguished from other dermal tumors for proper treatment. Diagnosis can be made with membranous CD99, PAS, and negativity for IHC of other tumors. Since DPE are superficial, of older age, female predominance, better behavior, and indeterminte molecular data compared with PE in deep anatomic sites, either there is specific technical difficulty with small DPE, an unrecognized molecular finding possibly involving Fli-1, or DPE might best be otherwise classified.

357 Evaluation of Angiogenesis Using VEGF, Microvessel Density and Trombospondin-1 in Cutaneous Malignant Melanomas

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Background: Angiogenesis has a role in the progression of various tumors including malignant melanoma. Angiogenesis has been assessed by microvessel density (MVD) using CD105, a marker which is more specific for tumor associated vessels. Vascular endothelial growth factor has also been associated with increased angiogenesis as well as tumor growth in tumors. Trombospondin -1 has an inhibitory role on tumor angiogenesis. In this study, VEGF and trombospondin-1 protein expression and MVD counts using CD105/endoglin antibody has been evaluated and correlated with histological prognostic variables in melanomas.

Design: Thirty nodular, 27 superficially spreading, 13 lentigo and 7 acral lentiginous cutaneous malignant melanomas were included . Immunohistochemical staining was performed for VEGF, CD105 and trombospondin-1 on formalin fixed and paraffin fixed tissues. VEGF expression was semi-quantitatively evaluated using staining intensity and proportion in tumor cells. MVD in the hot spots was estimated using the CD105 antibody. Trombospondin-1 protein expression has been immunohistochemically assessed in tumor cells as well as stromal cells using staining density.

Results: Positive staining for VEGF in the cytoplasm of tumor cells was present in 47 patients with different staining density and proportion. Staining density and staining proportion of VEGF were positively correlated with MVD assessed with CD105/endoglin antibody. VEGF expression was also correlated with tumor thickness, tumor growth phase and angiolymphatic invasion, but stage of the tumor were not related with staining density and proportion of VEGF. Using the endoglin antibody, median MVD was 30.2 microvessels per mm^2 (range 3-126). MVD was correlated with ulceration, Clark's level of invasion, mitotic index, lymphoid response and stage of the tumor. Subtypes of melanomas in this study did not differ with regard to VEGF expression and MVD counts assessed by CD15/endoglin expression. Trombospondin-1 protein expression was correlated with VEGF expression and MVD counts by endoglin expression in only tumor cells but not in stroma of the tumors. Trombospodin-1 expression was not related with prognostic variables studied.

Conclusions: These data suggest that angiogenesis as assessed by MVD counts using endoglin/CD 105 antibody, has been related to histological prognostic variables in melanomas. VEGF and trombospondin-1 has been involved in tumor angiogenesis in malignant melanomas.

358 The Role of FNA and Surgical Biopsy in the Evaluation of Scalp Lesions

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Background: Although the literature on surgical diagnoses of scalp dermatopathology is plentiful, information on the use of fine-needle aspiration (FNA) in diagnosing scalp lesions is sparse. Additionally, comprehensive reviews comparing surgical and FNA diagnosis of scalp lesions is scanty. The present study addresses the roles of surgery and FNA in management of patients with scalp lesions.

Design: 1362 surgical cases and 106 FNA's of scalp lesions from 1985-2005 were reviewed. Each patient's age, sex and previous medical history were recorded.
Results: Of the 1362 surgical cases reviewed, there were 746 male and 616 female patients ranging in age from 1 day old to 99 years of age. There were 455 cysts (33.4%); 203 nevi (14.9%); 136 keratoses (10.0%); 88 dermatoses (6.5%); 35 verrucae (2.6%); 132 primary benign tumors (9.7%); 148 primary malignant tumors (10.9%); 63 metastases (4.6%); 26 cases of alopecia (1.9%); and 76 other/miscellaneous (5.6%). Of the 106 FNA's, there were 66 male and 40 female patients, ranging in age from 26 to 91. Eighty-two patients (77.4%) had a previous history of malignancy. Of these aspirates, 80 were neoplastic and showed cells consistent with the patient's previous known primary. One case was an abscess, and the remaining case was unsatisfactory. The most common primary tumor to metastasize to the scalp was lung (25 cases), followed by hematopoietic malignancies (21 cases), melanoma (7 cases), head and neck (6 cases), sarcoma (5 cases), gastrointestinal (5 cases), renal (4 cases), prostate (3 cases), breast (2 cases) and meningiomas (2 cases). The remaining 24 aspirates were from patients who did not have a previous history of malignancy. These include 9 epidermal inclusion cysts, 6 lipomas, 2 large cell lymphomas and 1 case each of neurofibroma, hemangioma, adenocarcinoma, squamous cell carcinoma, melanoma, and angiosarcoma. The last case was unsatisfactory.
Conclusions: Striking differences were identified after comparison of the types of scalp lesions diagnosed with histologic versus cytologic examination, with 81.4% of tissue biopsy specimens that were benign (n=1151), contrasted with only 17.0% of FNA specimens (n=18), p<0.00001. This variation may be attributed to the referring physician or patient's history. Awareness of this fact can be useful to dermatologists or oncologists in selecting the better diagnostic procedure for a given patient.

359 Cellular Neurothekeoma: Analysis of 128 Cases

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Background: Cellular neurothekeomas are distinctive benign cutaneous tumors of uncertain histogenesis. As relatively few cases have been reported, their clinical and morphologic spectrum remain incompletely defined, and the significance of atypical histologic features is uncertain.
Design: To characterize these tumors further, 128 cellular neurothekeomas were retrieved from the authors' consult files. H&E sections were re-examined, immunohistochemistry was performed, and clinical details were obtained from referring physicians.
Results: There was a 1.8:1 female predominance, with a mean age of 24 yr (87% <40). Mean tumor size was 1.1 cm (range: 0.3-6 cm; 90% <2 cm). The tumors arose most often on the upper limb (33%) or head & neck (32%). Half the tumors were limited to the dermis, and half also involved subcutis. In 30% of cases, neurothekeoma was suggested by the referring pathologist; the most common other diagnoses offered were plexiform fibrohistiocytic tumor, benign fibrous histiocytoma, and low grade sarcoma. Histologically, most cases were poorly marginated; 33 (26%) infiltrated fat, and 10 (8%) entrapped skeletal muscle (all but 1 situated on the face). Nearly all tumors had a lobulated architecture and were composed of nests and bundles of epithelioid to spindled cells with palely eosinophilic cytoplasm, often separated by dense hyaline collagen; 17 (13%) showed focally sheet-like areas, and 5 (4%) were notably plexiform. Myxoid stroma was observed in 37 (29%) tumors; 11 (9%) were predominantly myxoid. Five (4%) showed marked stromal hyalinization. Osteoclastic giant cells were seen in 20 (16%) cases. Mean mitotic rate was 3 per 10 HPF; 27 (21%) had ≥5 per 10 HPF. Most tumors showed mild cytologic atypia in the form of nuclear variability and small nucleoli; 31 (24%) contained notably pleomorphic cells. All tumors were reactive for NKI-C3, 93/104 (89%) expressed NSE, 70/121 (58%) showed at least focal staining for SMA, and only 1 was desmin positive. All tumors were S-100 negative. Follow-up ranged from 5-146 months (mean 43). Nine tumors recurred locally (7 situated on the face), after a mean of 15 months; tumor had involved excision margins in all cases with available information.
Conclusions: Cellular neurothekeomas have a predilection for the upper limbs and head & neck of pediatric and young adult females and rarely recur following incomplete excision. There is no good evidence that these lesions show nerve sheath differentiation. Atypical histologic features (including pleomorphism, infiltration of subcutis, and a high mitotic rate) seem to have no clinical significance.

360 ACE Expression in Primary Melanoma: Correlation with Prognostic Parameters and Outcome

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Background: Angiotensin converting enzyme (ACE) is a protein that cleaves a His-Leu dipeptide from Angiotensin 1 to create Angiotensin 2. It is present in its highest concentration in the lungs, but is also located in plasma and in endothelial cells in various vascular beds throughout the body. ACE gene alterations have recently been implicated as conveying an increased risk for oral precancerous lesions in non-smokers, while ACE inhibitors have been reported to protect against cancer in patients. We immunostained primary and metastatic melanomas to determine frequency of ACE expression and association with prognostic parameters and outcome.
Design: Using fixed tissue microarray technology, 49 primary melanomas (PM), 12 metastatic melanomas (MM), and 6 benign nevi were immunostained for cytoplasmic ACE expression using kidney positive controls. Clinicopathologic parameters and follow up were obtained.
Results: 12 (24.5%) PM, 3 (25%) MM and 0 (0%) benign nevi expressed ACE. Significantly more (p=0.044) ACE-positive (82%) than ACE-negative (46%) PM metastasized. Overall survival tended to be worse in patients with ACE expressing PM (p=0.0791). Poor prognostic parameters (Clarke's Level IV and V [67% v 51%], Breslow's mean depth of invasion [4.2 v 3.6mm], mean mitoses per square mm [7.9 v 5.8], lymph node involvement [36% v 20%], Stage 3 and 4 [75% v 45%], and recurrence [18% v 7%]) were more frequent in ACE-expressing than non-expressing PM, although not reaching statistical significance. Age, sex, and frequency of ulceration were similar in both groups.
Conclusions: ACE expression is present in 25% of primary and metastatic melanoma, but not in benign nevi. ACE expression correlates significantly with higher rates of metastases and tends to correlate with poorer overall survival. It is associated with poor prognostic parameters (depth of invasion, mitoses, stage, recurrence) although not significantly so. ACE inhibitors, found to protect against cancer in patients treated for hypertension, may have therapeutic relevance in patients with ACE-expressing primary melanoma.

361 Detection of Human Herpesviruses in Pityriasis Rosea Skin Biopsies

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Background: Pityriasis rosea (PR) is a self-limited papulosquamous skin eruption most commonly seen in adolescents and young adults of unknown cause. Features of PR that suggest a viral etiology include clustering of cases and prodromal symptoms of headache, fever, arthralgia, and malaise. Findings of herpesviral-like particles by electron microscopy, and positive serology and PCR positivity for HHV-6 and HHV-7 have led some investigators to conclude that PR is due to HHV-6 and/or HHV-7 infection. However, since other investigators have been unable to detect these viruses in PR tissues the issue of the role of herpesviruses in the etiology of PR remains unresolved.
Design: We have examined FFPE skin biopsy tissues from 13 cases of histologically-confirmed cases of pityriasis rosea for the presence of all eight human herpesviruses by real-time multiplex PCR (55 cycles) with Taqman probes. DNA from each biopsy was extracted and subjected to GAPDH (housekeeping gene) PCR to confirm the presence of amplifiable DNA in all 13 cases. The assay was designed to amplify herpesvirus products ranged in size from 57-120 bp to make it suitable for detection of the relatively shorter DNA fragments typically obtained from FFPE tissue. Positive control DNA was run simultaneously.
Results: Results of real time PCR for detection of human herpesvirus (HSV1, HSV2, VZV, EBV, CMV, HHV6, HHV7, HHV8) DNA in these pityriasis rosea skin biopsies were uniformally negative.
Conclusions: These PCR results are not supportive of a role for any known human herpesvirus, including HHV-6 and HHV-7, in the etiology of these cases of pityriasis rosea. False negative results are unlikely given the good quality of the DNA and high sensitivity of the assay. However, definitive evaluation of the role of herpesviruses in PR awaits future studies with lesional DNA extracted from fresh skin biopsies of a larger series of cases.

362 Melastatin (MLSN) Gene Expression in Normal Skin Melanocytes

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Background: Melastatin (MLSN) mRNA expression is an independent prognostic factor in primary cutaneous melanoma. In this study, we examined the range of expression in normal melanocytes from varied sites, ages and adjacent skin tumors with a non-radioactive chromogenic in situ hybridization (CISH) method.
Design: Sixty-seven normal skin samples were obtained from cosmetic, amputation, circumcision; and excision specimens containing benign and malignant lesions. They consisted of 10 acral, 17 anogenital/nipple skin, 5 extremity, 18 head and neck, and 17 trunk skin samples. The mean age of patients was 38years old, range of 1month to 88years. Adjacent tumors were present in 45 specimens; 30 benign (19 melanocytic nevi) and 15 malignant skin tumors (5 melanomas). MLSN mRNA and S100 protein expression were measured per 100 basal keratinocytes [labeling index (LI)].
Results: MSLN expression was identified only in epidermal and follicular melanocytes. Overall, MLSN LI was significantly lower than S100 LI (8.2±6 vs. 12.0±7, P=0.0001: with exception of anogenital skin where both LIs were equivalent. By site and decreasing LI, MLSN and S100 LIs were 12.2±6.6/11.6±7.2 on anogenital; 8.7±5.1/16.4±7.5 on head and neck; 6.2±5.8/11.5±5.5 on truncal; 5.7±3.8/7.2±5.7 on acral; and 2.0±1.8/7.2±3.2 on extremity skin samples. By age, MLSN LI decreased until 60years, than rose; whereas S100 LI continued to decrease: 11.0±5.7/13.1±8.5 for <20 years; 8.2±5.9/14.4±7.4 for 20≤ and <40years; 5.3±6/9.8.1±4.4 for 40≤ and <60years; and 8.8±4.9/8.9±5.9 for patients ≥60years (P=0.04, ANOVA). No differences for MLSN or S100 LI were found comparing skin adjacent to benign vs. malignant tumors vs. no tumors. However, both MLSN and S100 LIs were significantly higher in skin adjacent to non-melanocytic tumors than melanocytic tumors: 10.1±6.5/15.1±7.5 vs. 6.3±5.4/10.7±6.4 (P=0.04). Notably, MLSN LI was higher in skin adjacent to melanocytic nevi (6.8±5.7 vs. 4.2±4.4) whereas S100 LI was higher adjacent to melanomas (12.2±6.1 vs. 10.3±6.6).
Conclusions: By CISH, MLSN mRNA expression is specific for melanocytes as defined by S100protein expression; however, it is not expressed by all normal melanocytes. The greater MLSN LI found in anogenital skin and skin associated with melanocytic nevi may be related to activation of normal melanocytic behavior; lower MLSN LI adjacent to melanoma suggests attenuation of MLSN due to proximity of the melanoma environment or due to field cancerization effect.

363 Chromogenic In Situ Hybridization (CISH) Analysis of Melastatin (MLSN) Gene Expression in Melamocytic Proliferations

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Background: Loss of expression of melastatin (MLSN) as measured by radioactive in situ hybridization has been described only in vertical growth phase and metastatic melanomas. In this study, we examined MLSN gene expression by chromogenic in situ hybridization (CISH) methods in a spectrum of melanocytic proliferations.
Design: One hundred and sixty eight benign and malignant melanocytic proliferations (Spitz nevi, blue nevi, dysplastic nevi, congenital nevus, common acquired nevi,

melanoma in situ, invasive melanomas and metastatic melanomas) were evaluated for MLSN mRNA expression by CISH. Nuclei were scored as having intact (>50% nuclear area stained), partial loss (≤50% nuclear staining) or complete loss (no nuclear staining) of MLSN mRNA.
Results: Overall, MLSN mRNA expression was heterogeneous with both nevi and cutaneous melanomas showing wide variations in the number of melanocytes showing either partial or intact expression. Patterned down-regulation of MLSN mRNA with melanocyte maturation was identified in ~75% of Spitz nevi and congenital nevi. In a less than 20% of these cases, complete loss of MLSN mRNA occurred in the deep dermal melanocytes (type C melanocytes in congenital nevi). Loss of MLSN mRNA could also be identified in 25% of melanoma in situ. For invasive melanoma, increasing depth of invasion significantly correlated with increasing loss of MLSN mRNA (r=0.3, P=0.0003). Metastatic melanomas showed either loss (80%) or regional areas (20%) of partial MLSN mRNA expression.
Conclusions: Loss of MLSN mRNA cannot be used as a diagnostic marker of melanoma as it can occur in a subset of melanocytic nevi and its expression can be conserved in both thick melanomas and metastases. The finding of patterned down-regulation correlating with melanocyte maturation reveals MLSN gene interactions with melanocyte phenotype and stroma.

364 The Basophil Specific Antibody 2D7 Is Expressed by Local Mastocytosis but Not by the Systemic One

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Background: 2D7 is considered an antibody which recognizes specifically the basophil. However, this antigen can be expressed by mast cells in not well known settings. We have tested if this antibody is expressed in mast cells disorders, and if it could differentiate aggressive presentations against benign states of mastocytosis.
Design: An immunohistochemical study of 20 skin biopsies of local mastocytosis (*telangiectasia macularis eruptiva perstans* and urticaria pigmentosa) and 6 liver or bone marrow biopsies of systemic mastocytosis using the antibody 2D7 was applied using the amplification method (NovocastraÒ). Sixteen biopsies of urticaria and the basophil cell line KU812F were used as reference. All cases and the cell line were tested against c-kit (DAKOÒ , 1/100).
Results: We found an intense expression of 2D7+ in the infiltrate of all skin biopsies of urticaria pigmentosa. The basophil cell line showed also an intense immunostaining. By contrast, biopsies of systemic mastocytosis showed no immunostaining at all with 2D7. By other way, biopsies of local and systemic mastocytosis showed a similar strong expression of c-kit. The urticaria biopsies and the human basophil cell line showed strong immunostaining against both 2D7 and c-kit.
Conclusions: It is postulated that 2D7 is not a specific antibody to basophils, because it is also expressed by different proliferating mast cells disorders. We consider that mast cells express 2D7 antibody in a different way in local than in systemic mastocytosis.

365 Utility of Immunocharacterization and Molecular Evaluation in the Diagnosis of Cutaneous Lymphoid Hyperplasia

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Background: Cutaneous lymphoid hyperplasia (CLH) is a disease, which simulates clinically or/and histologically a lymphoma. A precise characterization of its clinical and anatomopathological features are needed.
Design: A retrospective study of 35 biopsies of 28 patients diagnosed of CLH, with a median of follow-up of 6 years. More relevant clinical data were recorded. The characterization of the infiltrate included a histological and immunohistochemical approach to test CD20, CD43, CD3, CD1a, CD4, CD8, CD68, CD30, Bcl-6, Bcl-2, S-100 protein and Ki-67, k and l chains. The gene rearrangement of the IgH was obtained by PCR and Gene Scan evaluation in all cases.
Results: Mean of 48 years (range 20-69). It was more frequent in females (2/1). In 9 patients previous allergic history was observed. Lesions were more frequently unique, located in face, back and EESS. Histologically, a heavy polymorphic or monomorphic infiltrate, predominantly lymphocytic, of non-centrocyte-like phenotype, with scarce or no atypia was observed. Four histological patterns were found. In three biopsies unique findings were observed, such as severe angiocentrism, neurotropism and perineural invasion. In 2 patients the lesions recurred and showed the same histology. Immunohistochemically, the lymphocytic infiltrate was characterized as B or mixed B/T cells. CD1a+ cells were very abundant in many biopsies. The proliferation index was <20% in all cases, both in the primary and the recidivant lesions. Molecularly, in 2 patients the CLH was monoclonal; in the rest was polyclonal. In 5 patients the lesions recurred 1-3 years after the diagnosis. In 3 patients, multiple adenopathies appeared which corresponded to hyperplastic lymph nodes. Only a patient with monoclonal CLH developed a lymphocytic B cell lymphoma (mediastinic), with no skin affectation, 6 years after. In all patients the CLH resolved with pharmacological treatment or radiotherapy.
Conclusions: The CLH is generally a polyclonal B/T benign lymphoproliferative process, occasionally recurrent, which can rarely be associated to extracutaneous lymphoma. Clonality study is a useful tool in the differential diagnosis with skin lymphoma.

366 Use of p63 Expression in Distinguishing Primary and Metastatic Cutaneous Adnexal Neoplasms from Metastatic Adenocarcinomas to Skin

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Background: p63, a recently identified homologue of the p53 gene, is believed essential in the epithelial development and is widely expressed in benign and malignant skin adnexal tumors. We and others recently demonstrated the value of p63 in distinguishing primary adnexal tumors from visceral adenocarcinomas metastatic to skin. We now evaluate the use of p63 expression in rare examples of paired metastatic and originating primary skin adnexal carcinomas to distinguish these from cutaneous metastases of internal adenocarcinomas.
Design: Immunohistochemical analysis for p63 was performed on formalin-fixed, paraffin-embedded archival tissue from 11 definite metastases of primary adnexal carcinomas: 4 eccrine, 2 apocrine, and 1 each of pilomatrical, sebaceous, hidradenocarcinoma, mucinous adenocarcinoma, malignant mixed tumor. 9 of 11 corresponding cognate primary tumors were available for staining. 14 previously characterized metastatic adenocarcinomas to the skin (12 from breast, 2 from gastrointestinal tract) were included. Monoclonal antibody (clone 4A4) against all p63 isoforms was detected by streptavidin-biotin-peroxidase technique and expression was scored positive in tumor nuclei as follows: zero (0) = < 5%; 1+ = 5-25%; 2+ = 26-75%; 3+ = > 75%. As previously proposed, greater than 25% positive cells was deemed significant.
Results: p63 was uniformly expressed in epidermal and adnexal basal cells, including eccrine myoepithelial cells. p63 expression in 8 of 9 available primary adnexal tumors was 2+ or 3+. One apocrine carcinoma showed 1+ staining and the stains for two primary tumors (apocrine and mucinous adenocarcinomas) were not available. 6 cases of metastatic adnexal carcinoma were 2+ or 3+, as were their associated primaries. 5 metastases from skin primaries showed staining of 1+ or 0, including two cases with reduced staining from 2+ in their primaries. All cutaneous metastases from internal adenocarcinomas were negative (score 0) for p63, as previously reported.
Conclusions: Analysis of p63 expression is useful for distinguishing primary adnexal tumors and their metastases from metastatic adenocarcinomas to skin. Metastases from adnexal carcinomas generally retain p63 expression similar to their associated primary tumors. Of interest is the lack of significant p63 staining in cutaneous apocrine and mucinous carcinomas.

367 Melanocytic Lesions on the Distal Lower Extremity Share Features of Dysplastic Nevi and Melanoma In-Situ

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Background: Melanocytic lesions in certain locations such as the ankle may have atypical histological and clinical features stimulating melanoma. However the significance of such lesions and there relation to dysplastic nevi and melanomas is not clear.
Design: 112 melanocytic lesions located on the ankle were retrieved from 1990-2005. The cases were divided into melanocytic nevi (MN), Dysplastic nevi (DN), invasive and in situ melanomas (MM) and atypical melanocytic lesions (AML, cases that did not readily fit in any of the previous categories). Clinical and histological data were documented. Architectural features included: circumscription, symmetry, cohesiveness of nests, suprabasal melanocytes, confluence, and single cell proliferation. Cytological features included: round/euchromatic nuclei, nuclear enlargement, cell enlargement, and prominent nucleoli. Each criterion was given the value of 0 or 1, and the summation score was obtained for both architecture and cytology in each case of DN and AML. The presence of melanin in the stratum cornum was classified as diffuse (D), columnar (C) and absent (A). Inflammation was divided into absent (A), non brisk (NB) and brisk (B). Follow up was collected for all AML.
Results: Clinical and histological features are summarized in tables 1 and 2. The highest number category was melanoma probably due to referral biases in our practice. Follow-up of all cases , ranges between 3-13 years, by re-excision or clinical observation, shows no recurrence or progression.

	Cases Number	Mean Age (years)	Females %	Males %
MN	17	49.5	59	41
DN	32	51.4	60	40
MM	50	56.5	64	36
AML	9	47	78	12

	Architectural Atypia %			Cytological Atypia %			Inflammation %			Melanin %		
	Mild (0-1)	MOD (2-3)	SEV (4-6)	MILD (0-1)	MOD (2)	SEV (3-4)	A	NB	B	A	COL	DIF
DN	12.5	75	12.5	56	37.5	6.5	65	35	0	25	25	50
AML	0	44	56	56	22	22	56	44	0	44	22	34

Conclusions: This study highlights a group of melanocytic lesions located on the ankle which shares histological features with acral nevi, dysplastic nevi and melanoma. These melanocytic lesions are more predominant in females characterized by having moderate to sever architectural atypia and mild cytological atypia. After complete excision, follow-up indicates an apparently benign outcome of such lesions.

368 Differenting Spitzoid Melanoma from Spitz Nevus through CD99 Expression

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Background: The ambiguity between the histologic determination of Spitz nevus (SN) and spitzoid malignant melanoma (sMM) continues. Even though numerous

studies aimed at better defining the distinction between these two entities have been performed, a true diagnostic marker remains elusive. Previous studies have investigated the expression of cell markers such as MART-1, MIB-1 and bcl-2, as well as multiple chromosomal analyses. One cell marker that has yet to be studied in this quest is CD99, a transmembrane glycoprotein involved in cell apoptosis, adhesion, extravasation, and transmigration and thought to play a role in many neoplastic processes. CD99 expression in primary melanoma has recently been studied at our institute, demonstrating 61% of these cases expressed CD99. This study led to the evaluation of CD99 expression in these two previously difficult to differentiate melanocytic entities: sMM and SN.

Design: All cases of melanoma diagnosed in the past five years at our institute that included key words in the microscopic description: spitz, spitzoid, spindle spindled, epitheliod, and/or abundant eosinophilic cytoplasm, were included. Additionally, all cases of SN over the past seven years were retrieved. Each case was stained with anti-human CD99. Observed membranous staining was recorded as positive or negative, strong or weak, and diffuse or focal.

Results: Our search resulted in 28 cases of sMM and 74 cases of SN. Fourteen cases (50%) of sMM expressed CD99 compared with only four cases (5.4%) of SN. The various staining patterns of all cases are shown in Table 1.

Conclusions: As distinguishing SN from a sMM has significant consequences, considerable efforts have been made to assist in this effort. Despite numerous studies attempting to find a single differentiating marker, none have been entirely successful. This study shows that 50% all sMM express CD99, 7 of which demonstrated strong diffuse staining, while only 5% SN are positive and none showed strong diffuse staining patterns. While the exact role that CD99 plays in these melanocytic processes is unclear, this study does show that CD99 is an additional tool in distinguishing between sMM and SN and validates further studies into the prognostic significance of this protein expression.

CD99 expression patterns in sMM and SN

CD99 staining pattern	sMM expressing CD99 (n=28)	SN expressing CD99 (n=74)
strong difuse	25%	0%
strong focal	4%	4%
weak diffuse	11%	1%
weak focal	11%	0%
none	50%	95%

369 Increased Expression of Stem Cell Markers in Malignant Melanoma

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Background: The potential role of stem cells in neoplasia is a subject of recent interest. Three markers of melanocytic "stem cells" have recently been described. CD166 is expressed on the surface of mesenchymal stem cells and has been found on human melanoma cell lines. CD133 is expressed on the surface of dermal derived stem cells and is capable of differentiating into neural cells. Nestin is a intermediate filament expressed in the cytoplasm of neuroepithelial stem cells. This study was designed to assess whether these markers identify differences in melanocytic cells comprising banal nevi, primary melanoma, and metastatic melanoma.

Design: 5 micron sections of tissue microarrays containing normal tissue and 64 melanocytic lesions (19 banal nevi, 17 in-situ and invasive melanomas, and 28 metastatic melanomas) were mounted on covalent slides. Monoclonal antibodies for CD166 (BD clone 3A6), CD133 (Miltenyi Biotec clone AC133), and nestin (Chemicon clone 10C2) were assayed using standard ABC immunohistochemical technique with red alkaline phosphatase substrate. Slides were reviewed by two pathologists and lesions were scored as negative or positive if >10% of cells were stained. Fisher's exact test was used for group comparisons.

Results: The results are tabulated in the table below:

	Nevus	Primary Melanoma	Metastatic Melanoma
CD166 pos/total (%)	5/19 (26%)	11/17 (65%)	19/28 (68%)
CD133 pos/total (%)	7/19 (37%)	14/17 (83%)	21/28 (75%)
Nestin pos/total (%)	10/19 (53%)	11/17 (65%)	26/28 (93%)

A significantly greater percentage of melanomas (combined primary and metastatic) contained cells that expressed CD166 (P = 0.005), CD133 (P = 0.003), and nestin (P = 0.03) than banal nevi. Only nestin showed a statistical difference when comparing primary and metastatic melanoma (P = 0.05). A stepwise increase in the proportion of lesions expressing all three markers was observed from banal nevi (2/19) to primary melanomas (8/17) to metastatic melanoma (19/28), P = 0.0005. All cases of metastatic melanoma expressed at least one stem cell marker.

Conclusions: The increased expression of CD166, CD133, and nestin in melanoma suggests that progression to malignant melanoma likely involves genetic pathways instrumental to stem cell biology and normal tissue development. Further studies and characterization of these pathways may also reveal new prognostic markers for a disease whose prognosis in advanced stages is dismal.

370 Identification of Cutaneous Mast Cells with the Ziehl-Neelsen Stain

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Background: The biology of mast cells (MC) in immune response, tumor progression and vascularization is extensively studied; however the evaluation of MC in diagnostic pathology is limited due to their difficult identification with H&E. The Ziehl-Neelsen stain (ZNS) is routinely used to detect acid fast bacteria. However, it is little known that the ZNS is also helpful to identify MC and other cellular structures.

Design: We randomly selected 50 human cutaneous inflammatory cases and performed MC counts with the ZNS, which stains MC cytoplasmic granules dark blue. MC counts were done blindly, without access to clinical or pathologic diagnoses, in the 40x area where MC were most concentrated. Eosinophils (Eo) were counted similarly with the H&E stain.

Results: The ZNS method to identify MC was validated with Giemsa and with CD117 in selected cases including cutaneous mastocytoma (not listed in table) and in non-inflamed control tissue. The inflammatory cases included dermatitis (e.g. contact, atopic, dyshidrotic, Darier, psoriasis, granuloma annulare, neurodermatitis, sarcoidosis), alopecia (e.g. areata, androgenetic, scarring), environmental and infectious (e.g. drug, arthropod reactions, scabies, molluscum, wart, abscess, etc). See table.

MC and Eo counts/hpf expressed as averages.

Disease category	n	MC	Eo
Spongiotic dermatitis	14	7.1	12.8
Other dermatitis	13	8	0.4
Alopecia	6	9.2	0
Environmental	8	7.5	6.6
Infections	5	9.2	10.2
Normal control	4	6.5	0

The MC counts remained rather stable in all categories, with a range of 6.5 to 9.2/hpf. There was a variable relationship of MC counts with Eo counts; and expected tissue eosinophilia in allergic and environmental disorders. Cases with increased Eo (n=22) had MC counts of 8.5/hpf, while cases without Eo (n=28) had 7.3/hpf.

Conclusions: The ZNS is an inexpensive and readily available method that allows easy MC identification. This initial evaluation in dermatopathology samples indicates that there appears to be limited variation of MC counts in a wide variety of cutaneous tissue responses. This is the first description and validation of this method in diagnostic dermatopathology.

371 CD99 Immunoreactivity in the Evolution of Mycosis Fungoides

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Background: CD99 is a transmembrane protein that is expressed on the majority of hematopoietic cells and all peripheral blood T cells. Although its function is not completely understood, CD99 has been implicated in homotypic cell adhesion, diapedesis, apoptosis, and protein transport. CD99 expression in the lymphocytes of mycosis fungoides (MF), a cutaneous T-cell lymphoma, was assessed to determine whether CD99 positivity is altered in the malignant transformation. To our knowledge, no similar study has been done before.

Design: We examined 15 cases of MF for CD99 immunoreactivity; 9 patch/plaque stage and 6 tumor stage MF. These were compared to the CD99 expression in 8 reactive dermatoses. The specimens were evaluated for percentage of lymphocytes expressing CD99.

Results: Our data show 8/15 cases had <25%, 3/15 had 50 – 75% and 4/15 had >75% of the lymphocytes showing CD99 expression. All 6 cases of tumor stage MF had very low to no CD99 expression.

Conclusions: This study suggests an association between CD99 expression and MF evolution from plaque to tumor stage. Implications about prognostic significance are premature in this small study. However these findings warrant further investigation.

Percent of lymphocytes expressing CD99

MF subtype	0-25%	25-50%	50-75%	75-100%
Patch/plaque (n=7)	2	0	2	3
Tumor (n=6)	6	0	0	0
Folliculocentric (n=1)	0	0	1	0
Granulomatous (n=1)	0	0	0	1
Reactive dermatosis (n=8)	0	0	0	8

CD99 expression in the lymphocytes of mycosis fungoides in various stages of evolution and reactive cutaneous dermatoses

372 "HPV Vulvitis" Revisited: Detection of Epidermal Verruciformis Associated HPV Types

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Background: The putative diagnosis "Human papillomavirus (HPV) vulvitis" describes a group of woman presenting with vulvar pruritus, pain or dyspareunia who are suspected to have HPV infection by clinicopathologic findings, which frequently included vestibular squamous papillomatosis (VSP) and vulvar erythema (VE). Prior studies of genital-mucosa HPV in HPV vulvitis have been negative; however, the presence of other HPV types such as epidermodysplasia verruciformis (EV) and cutaneous (CU) types has not been studied.

Design: In the current study, the presence of HPV DNA in the paraffin embedded specimens from women with symptomatic VSP (n=14) and VE (n=13) and controls of solitary vulvar fibroepithelial polyps (FEP) (n=15), condyloma (n=10), and normal vulva skin (n=10) was investigated using polymerase chain reaction using 15 sets of primer pairs to amplify most CU, GM and EV genotypes. Specific HPV types was determined by direct sequencing.

Results: HPV DNA was detected in 40/55 (73%) VSP/VE specimens (20% had multiple HPV infections), 7/15 (47%) FEP, 10/10 condyloma, and 1/10 normal vulva skin. EV HPV types were significantly more frequent in VSP/VE than controls (P<0.05). CU, GM and EV HPV genotypes were 0%, 17% and 73% for VSP/EV, 0%, 50% and 50% for FEP and 9%, 91% and 0% for condyloma. Histologically, the presence of HPV DNA significantly correlated for koilocytes fulfilling Meisel's criteria (P< 0.05 compared with absence of HPV) among VSP/EV and FEP groups. The presence of group B, subgroup A EV HPV types (alb-1, alb-4, and alb-7) was detected significantly more frequently in symptomatic VSP/VE patients than those without symptoms (80% vs. 16%, P< 0.05).

Conclusions: The higher frequency of HPV, predominantly EV, subgroup A genotypes, in patients with VSP/VE and the correlation of clinical symptoms with subgroup A of EV HPV suggesting that the condition "HPV vulvitis" may indeed exist.

373 The Predictive Value of Lymphatic Invasion Detected by D2-40 Immunostain in the Malignant Melanoma Patients with Positive Lymph Nodes

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Background: Metastasis to the regional lymph nodes in patient with malignant melanoma carries a significantly worse prognosis. However, there is no reliable clinical or histologic features to predict regional lymph node metastasis in malignant melenoma, since metastasis may not always be clinically or radiologically evident. Although lymphatic invsion may increase the incidence of regional lymph node metastasis, there is limited data in the literature addressing this correlation. Furthermore, identification of lymphatic invasion may not be easy in routine histologic evaluation since tissue retracting artifact, a common finding in the malignant melanoma, can simulate tumor lymphatic invasion. In this study, we investigated if lymphatic invasion detected by a specific and sensitive immunohistochemical lymphatic marker, D2-40, can predict regional lymph node metastasis in patient with malignant melanomas.

Design: 22 cutaneous malignant melanoma negative for lymph node metastasis and 8 cutaneous malignant melanoma positive for lymph node metastasis were retrieved from the hospital computer system. Immunostaining for D2-40 was performed on an automated immunostainer with appropriate positive and negative controls. The cases positive for lymphatic invasion were counted and Chi-Square method was used for statistical analysis.

Results: Lymphatic invasion by neoplastic cells detected by D2-40 was observed in 7/8 (87.5%) patients with positive lymph nodes, four of which was observed on H and E stained slides. 1/22 (4.5%) patients with negative lymph nodes was found to have D2-40 positive lymphatic invasion by the neoplastic cells, which was not observed on H and E stained slide. The D2-40 confirmed lymphatic invasion was significant higher in the patients with lymph node metastasis than those patients with negative lymph nodes in the control group ($P < 0.01$).

Conclusions: Our results indicated that the incidence of lymph node metastasis is significantly increased in malignant melanoma patients with positive lymphatic invasion detected by D2-40 immunostaining. Lymphatic invasion detected by D2-40 in malignant cutaneous melanoma may be one of the useful parameters in selecting melanoma patients for regional lymph node resection. In addition, D2-40 should be used to confirm lymphatic invasion for suspicious cases on H and E stained slides.

374 Different Immunologic Microenvironments in Halo Nevi and Melanomas

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Background: Regression is present in halo nevi and melanomas. In halo nevi, the nevus cells get destroyed with the passage of time. Although focal regression occurs in some melanomas, complete regression is rare even with brisk lymphocytic infiltrate. Perforin-mediated cytotoxicity is a key mechanism for CD8+ T cell-mediated effector function. Recently, Foxp3 expressing CD4+/25+ T cells have been shown to inhibit tumor rejection and tissue inflammation by suppressing the function of CD8+ T cells. We compared the immunologic microenvironments in these entities in order to understand better the regression mechanism in these lesions.

Design: 15 halo nevi, 5 dysplastic nevi with halo reaction and 20 melanomas with brisk lymphocytic infiltrates were analyzed. We stained for CD4, CD8, CD25, Foxp3, and perforin. T cells were counted manually.

Results: T cells in close contact with tumor cells were counted as group A; and the rest of the T cells were counted as group B. In early regression of nevi, the ratios of CD8+/CD4+ were 3/1 and 2/1 for groups A and B; Foxp3+/CD4+ was 1/2 for both groups. Perforin+ T cells counted 60% and 30% of the CD8+ cells of groups A and B. In late regression of nevi, the ratio of CD8+/CD4+ decreased to 1 for both groups; Foxp3+/CD4+ was 1. In melanomas, CD8+/CD4+ ratios were similar to those of nevi in early regression stage; but Foxp3+/CD4+ was 1/15. However, perforin+ T cells counted less than 5% of CD8+ T cells in melanomas. CD25+ T cells were mildly more than Foxp3+ T cells.

Conclusions: The majority of tumor-infiltrating CD8+ T cells express perforin, suggesting that perforin-mediated cytotoxicity may be the key mechanism involving the early regression of halo nevi. Increased Foxp3+ T regulatory cells most likely reflect the negative feedback mechanisms to prevent tissue damage from severe immunoreaction in halo nevi. Although CD8+ T cells significantly increase in melanomas similarly to in halo nevi, these T cells lack perforin, suggesting defective CTL around tumor, which may contribute to the mechanisms of immune evasion leading to the aggressive growth of the tumor. The failed immunoreaction in melanomas does not cause tissue damage, which may reflect to the paucity of Foxp3+ T cells in melanomas. It would be intersting to study what mechanisms inactivate effector CTL independant of Foxp3+ T regulatory cells in the future.

375 Immunohistochemical and Molecular Analysis of Dermatofibrosarcoma Protuberans. A Study of 75 Cases

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Background: Dermatofibrosarcoma protuberans (DFSP) is a rare, infiltrative skin tumour of intermediate malignancy, with a high rate of recurrence. This tumour presents specific cytogenetic features such as reciprocal translocations t(17;22)(q22;q13.1). As a consequence of this rearrangement, fusion of the collagen type I-alpha 1(COL1A1) gene, on 17q, with the platelet-derived growth factor beta-chain (PDGFB) gene, on 22q, is observed. The purpose of this study is to characterize, for the first time, the immunohistochemical profile and the presence of the COL1A1-PDGFB gene fusion of a large series of DFSP.

Design: Paraffin-embedded material from 75 diagnosed DFSP were collected for the immunohistochemical analysis of the following markers: CD34, factor XIIIa, APO-D, stromelysin III, PDGFR-beta, PDGFR-alpha, c-KIT and p53. A multiplex reverse transcriptase-polymerase chain reaction (RT-PCR) was carried out on the same cases using specific primers that flank the whole exons of the COL1A1 gene and the exon 2 of the PDGFB. Sequencing analysis was carried out to characterize the specific breakpoints.

Results: Most of the analyzed cases were conventional DFSP, expressing high levels of vimentin (100%), stromelysin (97%), CD34 (91%), PDGFR-alpha (91%) and PDGFR-beta (91%). Apo-D was present in 48% of cases, whereas factor XIIIa, p53 and c-KIT were detected in 11%, 5% and 1% of cases respectively. We obtained RNA of sufficient quality for the molecular studies in 55 cases, from which COL1A1-PDGFB fusion was confirmed in 36 cases. In the majority of cases, fusion occurs with exons between 25 and 48 of COL1A1 and exon 2 of PDGFB. Interestingly, we found novel breakpoints between COL1A1 and PDGFB that have not so far been described before.

Conclusions: Our study represents a preliminary analysis of the variability of the COL1A1-PDGFB fusion genes and the immunohistochemical profile of 75 DFSP cases with the final aim of clarifying the clinicopathological significance of the COL1A1-PDGFB fusion types in the pathogenesis and prognosis of this peculiar neoplasm. Supported with FIS grant PI040822 from the Ministry of Health, Madrid, and by the Conselleria de Educacion y Ciencia, Valencia, Spain.

376 The Expression of CD23 in Malignant Lymphoma of Follicular Center Cell and Marginal Zone Subtypes

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Background: CD23 expression in normal B lymphocytes is limited to autoreactive B cells, mantle zone lymphocytes and has been described in mature B cells manifesting an activated phenotype. In the context of CD23 as a marker of hematologic dyscrasia/malignancy, expression of this marker is associated with small lymphocytic lymphoma and with chronic lymphocytic leukemia (Koiso).

Design: An absence of CD23 expression within small lymphocytes is held to be a feature observed in cutaneous marginal zone lymphoma. Its expression by neoplastic lymphocytes in primary cutaneous B cell lymphoma is not described. This paper describes 9 patients with cutaneous B cell lymphoma in whom CD23 expression was observed amidst the neoplastic B cell populace. The significance of CD23 expression is explored. The addition the expression of CD40, an important regulator of CD23 expression, was also evaluated.

Results: Three patients had recurrent primary cutaneous marginal zone lymphoma. Two patients had primary cutaneous follicle center cell lymphoma, one patient had recurrent marginal zone lymphoma secondarily involving the skin and 2 patients had primary cutaneous marginal zone lymphoma without recurrence. All cases showed a dominance of CD23 expression; positivity was in the context of both transformed and small lymphocytes. Concomitant expression of CD40 was observed in the zones of CD23 expression.

Conclusions: CD23 expression amidst cutaneous B cells should not always be equated with benignancy. CD23 is also an anti-apoptotic activation marker and may define a marker associated with a more aggressive behavior at least in the context of recurrent local disease. CD23 is a precentroblast markers however it is also upregulated in cell sundergoing blastic transformation. The basis is one related to CD40 expression CD40 is held to be antiapoptotic being induced by nuclear factor kappa beta. This pilto study suggests that CD23 may correlated with disease progression in the setting of cutaneous B cell lymphoma

377 Cutaneous Involvement in T Prolymphocytic Leukemia

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Background: T prolymphocytic leukemia, formerly categorized as T cell chronic lymphocytic leukemia, is a rare and aggressive hematologic malignancy. The skin is disproportionately involved compared to other organ systems and relative to other primary extracutaneous hematologic dyscrasias.

Design: The clinical and pathological features of 5 patients presenting with skin involvement in the setting of T prolymphocytic leukemia are discussed. In each of these cases the skin disease was a cardinal manifestation of the intitial presentation.

Results: The patient population comprised two women and three men, mean age of 60 years. The disease was heralded in all by significant skin involvement with facial preference involvement, edema, purpura and lesional symmetry being characteristic. Five cases of cutaneous T prolymphocytic leukemia are presented from a clinical, light microscopic and phenotypic perspectiveThe skin biopsies demonstrated a largely non-epidermotropic lymphocytic infiltrate in an angiocentric array accompanied by hemorrhage. The cells were noncerebriform, showing irregularly blebbed nuclei with small nucleoli and eosinophilic cytoplasm. Phenotypic studies revealed three prevailing profiles: CD4 dominant in 3 2, CD8 dominant in one and co-expression of CD4 and CD8 in one. CD3 loss was seen in one case. Cutaneous lymphocyte antigen expression was weak or absent. Cytogenetic studies in one patient demonstrated an inverted chromosome 14. Three patients died from their disease within one year following diagnosis. One patient has had an indolent clinical course. One patient is lost to follow up. The paraffin embedded tissue examined in one case showed trisomy eight and c-myc overamplification. This patient was the youngest in the series and had a very aggressive clinical course.

Conclusions: T prolymphocytic leukemia is a distinctive form of post thymic T cell lymphoma showing unusual cutaneous tropism. While the clinical course may be

aggressive, a more insidious presentation may also be encountered although only rarely. Whether or trisomy 8/cmyc overamplification isolates more aggressive cases requires further investigation.

378 Cutaneous Vasculopathy and Sclerodermoid Reactions in Association with Cytomegalovirus Infection

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Background: Viruses have long been held to be of pathogenetic importance in the evolution of autoimmune connective tissue disease (CTD). Among the main viruses are B19, cytomegalovirus, hepatitis C and epstein barr virus. The mechanisms remain elusive and their specific role in autoimmune disease remains controversial.

Design: We describe 9 adults who developed cutaneous CTD stigmata in temporal association with recent cytomegalovirus (CMV) infection but without the classic cytopathic changes of CMV infection. The clinical features and pathological features are reviewed. As well in situ hybridization studies to assess for in situ CMV RNA transcript expression is explored.

Results: Nine adults were encountered with relatively sudden onset clinical presentations encompassing cutaneous vasculitis in five and widespread scleroderma in four. In all patients CMV DNA hybrid capture assays, blood cultures positive for CMV and or elevated IgM CMV antibodies were documented suggesting active CMV infection temporally associated with their autoimmune course. The vasculitic lesions included lymphocytic, neutrophilic and granulomatous vasculitis. While no CMV inclusions were seen, in situ hybridization studies revealed CMV RNA transcript expression with primary localization to the endothelium.

Conclusions: CMV can be associated with cutaneous scleroderma and small vessel vasculitis. The probable basis of the changes is abortive reactivation leading to the production of CMV associated early proteins. These proteins may in turn define antigenic targets for molecular mimicry and/or create a state of neoantigenicity by directly influencing gene expression, the latter primarily in the context of accelerated apoptosis, a sequelae of early protein expression.

379 Potential Therapeutic Utility of Epidermal Growth Factor Receptor (EGFR) and C-Kit Immunostaining, and Clinicopathologic Profile of Metastatic Basal Cell Carcinomas (BCC): A Study of 9 Cases

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Background: EGFR and C-kit are frequently being requested by oncologists for therapeutic considerations. We had received one such request in a patient with incurable metastatic basal cell carcinoma. This prompted us to evaluate the utility of EGFR and C-kit in a cohort of patients with metastatic basal cell carcinoma. We had also noticed, focal neuroendocrine-like features in one other case, and this led us to study for neuroendocrine differentiation using markers synaptophysin and chromogranin. This is a study of 9 cases with metastatic BCC.

Design: These 9 cases were included from three institutions. Representative H & E slides were available for review, and paraffin blocks of primary and/or metastases were obtained in 8 cases. EGFR and C-kit immunostaining was performed. Chromogranin and synaptophysin imunostaining was also performed.

Results: There was no site predilection for primary sites. Metastases was more likely in regional lymph nodes, and distant metastastic sites included bone, lung and kidney. All the primary tumors were deeply invasive, some extending into underlying skeletal muscle, and most cases exhibited infiltrative sclerosing growth pattern. EGFR was strongly positive in all cases. C-kit was uniformly negative in all cases. There was patchy focal positivity for one or both neuroendocrine markers in 6 out of 8 cases.

Conclusions: There is increased expression of EGFR in BCC, and inhibition of EGFR may provide a rational target for anticancer therapy, especially when traditional chemotherapy has failed or cannot be used. C-kit expression was lacking in all our cases; although there may be no therapeutic value to this result, it aids in differential diagnosis of metastatic BCC from metastatic adenoid cystic Ca, a close morphologic mimic. Focal neuroendocrine differentiation is seen in both the primary and metastatic BCC in a subset of cases, the significance of this finding needs to be further explored.

380 The Notch Signaling in Melanocytic Nevi and Cutaneous Malignant Melanoma

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Background: The Notch signaling has been implicated in the regulation of self-renewal of adult stem cells and differentiation of precursors along a specific cell lineage, in normal embryonic development and organogenesis. There is also evidence that signaling through Notch receptors regulate cell proliferation and cell survival in several types of cancer, with opposing results depending on tissue context. No data are available in the literature concerning modulation of the expression of Notch receptors, and their ligands, in human cutaneous malignant melanoma.

Design: The expression of Notch 1, Notch 2, Jagged 1, Jagged 2 and Delta-like 1 proteins, was investigated by immunohistochemistry in a series of benign and malignant human melanocytic lesions: 5 common melanocytic nevi; 5 atypical or "dysplastic" melanocytic nevi and 20 melanomas (5 *in situ,* 5 T1-T2, 5 T3-T4 and 5 metastatic melanomas). Human melanoma cells (A375) were transfected with a constitutively activated form of Notch 1. Transfected cells were evaluated for Protease activated receptor (PAR) -1 expression, cell migration and cell growth.

Results: We found that the expression of Notch 1 and Notch 2, as well as Notch ligands, was upregulated in atypical nevi and melanomas as compared with common melanocytic nevi. In human melanoma cells (A375) up-regulation of Notch 1 signaling determined down regulation of protease activated receptor (PAR) -1 expression, inhibition of cell migration and reduction of anchorage-dependent and independent cell growth.

Conclusions: Taken together our results indicate that upregulation of the Jagged-Notch ligand-receptor system may represent an early event in melanocytic tumor growth; however activation of Notch 1 - mediated intracellular signaling may favor a less malignant phenotype.

381 Cutaneous Peripheral T-Cell Lymphoma Associated with a Proliferation of Large B-Cells ("Large B-Cell Rich T-Cell Lymphoma")

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Background: Cutaneous peripheral T-cell lymphomas (CPTCL) represent approximately 1% of all cutaneous T-cell lymphomas (CTCL). While systemic peripheral T-cell lymphoma (PTCL) has been well described, little has been published regarding CPTCL.

Design: As follow up to an initial examination of systemic PTCL complicated by a proliferation of large B-cells, we studied 31 cases of CPTCL that contained numerous large B-cells. Histological, immunohistochemical, molecular, and clinical follow-up information was reviewed.

Results: All cases showed a mixed population of B- and T-cells, with many large cells in both lineages. The T-cells in particular showed a range in cell size. In 4/24 cases, light chain monotypia was present (3 kappa: 1 lambda). Molecular studies for T-cell gene rearrangements were positive in 26/27 cases. In contrast, molecular studies for immunoglobulin gene rearrangements were positive in only 4/23 cases. Of eighteen cases evaluated for the presence of Epstein-Barr virus, 6 were positive. Clinical follow-up information was available for 10 patients, with a mean duration of follow-up of 37 months (6-74 months). The mean age at presentation was 63.3 years (34-83). No gender predilection was noted (4 men: 6 women). The clinical presentation was varied and included cutaneous and subcutaneous nodules, plaques, maculopapular rash, and skin discoloration. Patients presented with either single or multiple lesions in all regions of the body, particularly the head and neck, trunk, and upper extremities (7/10). All patients had a complete clinical staging work-up, and systemic involvement was present in 5/10 cases. Chemotherapeutic regimens were pursued in 6 patients (5 with secondary and 1 with primary cutaneous involvement). The remaining patients with primary cutaneous involvement were treated with excision and observation (2/10), local radiation therapy (1/10), or topical/oral modalities (1/10). At last follow-up, 4/5 patients with systemic involvement were dead of disease (3) or treatment complications (1). The patients with primary cutaneous involvement were all alive (4 with clinical remission and 1 with persistent disease).

Conclusions: The presence of numerous large B- and T-cells caused initial misdiagnosis as reactive processes, or T-cell rich large B-cell lymphomas, in a subset of our cases. Careful histological examination, immunohistochemical analysis, and molecular studies for both T-cell and B-cell gene rearrangements will aid in the diagnosis of this unusual entity.

382 Her-2/neu Expression and Gene Amplification in Malignant and Metastasizing Skin Adnexal Tumors

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Background: We recently reported a metastasizing hidradenocarcinoma (HC) with strong membranous immunohistochemical staining for Her-2/neu and detection of *Her-2/neu* gene amplification by fluorescence in situ hybridization (FISH). We now stain 13 additional primary malignant skin adnexal tumors, 8 of which also gave rise to metastases, in order to examine the frequency of Her-2/neu expression.

Design: Cases were retrieved from the archival and consult files of 3 institutions. Cases included HC (3), eccrine (4), mucinous (2), microcystic adnexal (2), trichilemmal (2), and apocrine (1) carcinomas. H&E-stained slides were reviewed to confirm the diagnoses. Formalin-fixed, paraffin-embedded sections incubated with Her-2/neu antibody (NeoMarkers, Inc, Freemont, CA) were detected using the strepavidin-biotin-peroxidase method. In the one case of HC showing strikingly intense membranous staining, we utilized FISH to determine the amplification status of the *Her-2/neu* locus.

Results: In one case of HC, strong membranous staining for Her-2/neu was noted and FISH demonstrated significant amplification (2.5-fold) of the *Her-2/neu* locus on chromosome 17p. Of the remaining cases, two showed limited, weak membranous staining on immunohistochemistry for Her-2/neu, not typically suggestive of genetic amplification. The remaining 11 cases were entirely negative on immunohistochemistry. FISH was not performed on these weakly staining and negative cases.

Conclusions: Intense, membranous immunohistochemical staining with amplification of the *Her-2/neu* locus by FISH appear to be uncommon in primary cutaneous adnexal carcinomas, including more aggressive types that give rise to metastasis. In the one case of metastasizing HC we previously reported with demonstrable genetic amplification of *Her-2/neu*, the patient received trastuzumab adjuvant therapy in addition to chemotherapeutic and radiation treatment. In the rare cases of aggressive or metastatic cutaneous adnexal carcinomas, such studies could provide an additional therapeutic option that has been recently proven highly effacacious in breast carcinomas with similar levels of *Her-2/neu* amplification.

383 Expression of VEGF and Flk-1 in Malignant Melanoma

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Background: Angiogenesis is a very complex biological mechanism in which several growth factors cooperate to stimulate vascular proliferation in normal and neoplastic tissue. Vascular endothelial growth factor (VEGF) is an angiogenic promoter by stimulating cell proliferation, migration and enhancing vascular permeability. Its biological activities are mediated mainly by two tyrosine-kinase receptors: VEGFR2 or Flk-1/KDR and VEGFR1 or Flt-1. In melanomas, the data regarding the association between VEGF expression and vertical growth or progression is still controversial

Design: A total of 38 cases of malignant melanoma were evaluated by immunohistochemistry for the expression of VEGF (Neomarkers) and Flk-1 (Santa Cruz Biotech). Tissue cores from formalin-fixed, paraffin-embedded donor blocks were arrayed to create a tissue microarray block. Immunohistochemical results were evaluated assessing the percentage of positive melanoma cells, independently of the intensity of the staining. The results were correlated with several clinicopathological factors. For the statistical analyses we performed the chi-square test and Spearman correlation ($p<0.05$).

Results: 38 cases of malignant melanoma were evaluated: 16 patients were men and 22 women; age at the time of diagnosis ranged from 22 to 85 years (mean 52, SD 16.4). Tumors were located in skin of the back in 12 cases, head and neck in 4, chest and abdomen in 6, arms in 4 and legs 11 cases. Clark level,s were: II in 10 (26.3%) cases, III in 23 (60.5%) and IV in 5 (13.2%) cases. The Breslow thickness ranged from 0.20 to 4.00 mm (mean 1.59, SD 0.88mm); 24 (63%) cases had a Breslow thickness >1mm. Eight out of 26 cases showed ulceration. VEGF expression ranged from 0 to 100% (mean 28%, SD 35); 34% (13/38) of the tumors had >50% of positive cells. Flk-1 expression ranged from 10% to 100% (mean 50.2%, SD 25.8), and 54% (19/38) cases showed positivity in >50% of the cells. Tumors containing high levels of VEGF had also increased expression of Flk-1 ($r=0.535$, $p<0.001$), as well as in melanomas with higher Breslow thickness ($p=0.077$). However, we found no association between VEGF expression and age, sex, ulceration and Clark level,s (p=n.s.).

Conclusions: In the present series of melanomas, our results support that overexpression of VEGF is associated with higher Flk-1 levels and with more advanced disease.

384 Morphological Spectrum of Eccrine Porocarcinoma

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Background: Porocarcinomas are thought to be differentiated into the acrosyringeal portion of the sweat duct. They may develop as a malignant transformation of poroma or de novo. The latter cases may be a diagnostic challenge. Our aim is to evaluate the morphological spectrum and the clinicopathologic difference between the two above-mentioned types of porocarcinoma.

Design: We analyzed the clinicopathologic features of 21 cases of eccrine porocarcinoma. The ages of the cases ranged from 47 to 95 years(average 75 years). The male-to-female ratio was 1:2. In addition to the gross and histological findings based on HE sections, special stain(d-PAS) and immunohistochemistry with CEA, CK7, and 34βE12 were performed.

Results: The average size of the tumor was 2.2 cm. Grossly, 15 cases were of the pedunculated type, and 6 cases were of the sessile type. Histologically, an area of carcinoma adjacent to the component of eccrine poroma was categorized into malignant transformation of poroma, and was observed in 13 cases(62 %). The other 8 cases were diagnosed as de novo eccrine porocarcinoma. Ductal formations were noted in 9 out of the 13 cases(69.2%) in malignant transformation of poroma, and 3 out of the 8(37.5%) cases in de novo porocarcinoma. Atypical cuticular cells were seen in 11 cases (84.6%) in malignant transformation of poroma, and 6 cases(75%) in de novo porocarcinoma. Ductal formation was significantly observed in cases of transformation of poroma($P<0.05$), although no significant difference was seen in atypical cuticular cells between malignant transformation of poroma and de novo porocarcinoma. Epidermal endophytic growth with nodular and lobular proliferation was noted in 20 cases.

Conclusions: Eccrine porocarcinomas were divided into de novo porocarcinoma and malignant transformation of poroma. The latter showed a wider range of histologic patterns. The ductal formation was frequently noted in the latter cases. Growth pattern of epidermal endophytic growth with nodular and lobular proliferation seems to be a low-power-view clue to diagnose eccrine porocarcinoma.

385 Cytokeratin 7 and 20 Expression in Clear Cell Lesions of the Skin

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Background: Clear cell lesions of the skin can be difficult to distinguish histologically. We studied the utility of cytokeratin 7 and cytokeratin 20 expression in clear cell lesions of the skin, including metastatic renal cell carcinoma.

Design: Ninety-three cutaneous clear cell lesions including 15 sebaceous adenomas (SeA), 13 sebaceous carcinomas (SeC), 13 metastatic renal cell carcinomas (MRCC), 10 xanthelasmas, 10 xanthogranulomas, 9 xanthomas, 8 balloon cell nevi, 7 clear cell hidradenomas, 5 squamous cell carcinomas, clear cell type (SCC-CC) and 3 basal cell carcinomas, clear cell type (BCC-CC) were examined by immunohistochemistry for the expression of cytokeratins 7 and 20.

Results: Ten of the 15 (66%) SeAs expressed cytokeratin 7 in greater than 25% of the cells compared to 1/13 (7.7%) in the SeCs. The SeCs that expressed cytokeratin 7 were all from the orbit, including the eyelid and conjunctiva, while those from extra-orbital skin lacked cytokeratin 7 expression. Two of the 5 SeAs that did not express cytokeratin 7 were from a patient with Muir-Torre syndrome (M-TS). The remaining 3 patients had no history of M-TS. Two of the SeCs were from M-TS patients and did not express cytokeratin 7. Cytokeratin 7 was expressed in 7/7 (100%) of clear cell hidradenomas, all with greater than 25% of cells staining. Cytokeratin 7 was weakly expressed in 1/13 (7.7%) MRCC, moderately expressed in 1/3 (33%) BCC-CCs, and strongly expressed in 2/5 (40%) SCC-CCs. Xanthelasmas, xanthogranulomas, xanthomas, and balloon cell nevi had no expression of cytokeratin 7. Expression of cytokeratin 20 was negligible in all of the tumors studied.

Conclusions: Our study demonstrates that cytokeratin 7 may be useful in differentiating SeA from SeC, outside the setting of M-TS, since cytokeratin 7 is expressed more intensely and in a greater percentage of lesional cells in SeA than SeC. This finding is even more dramatic in extra-orbital lesions where no expression of cytokeratin 7 was found in the SeCs studied. Further analysis is required to clarify the apparent absence of cytokeratin 7 in sebaceous lesions of patients with MT-S.

386 Characterization of Cutaneous Perineuriomas by Immunohistochemistry

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Background: Perineuriomas are uncommon benign nerve sheath tumors that have been identified in the skin, soft tissue, and gastrointestinal tract. They are typically epithelial membrane antigen (EMA) positive and have characteristic ultrastructural findings. Prior studies have shown that EMA can be weakly positive or even negative despite characteristic ultrastructural findings. A majority of perineuriomas have also been shown to be CD34 positive and occasionally S100 positive. In this study, we further characterize cutaneous perineuriomas by immunohistochemistry and compare them to a neurofibroma control group.

Design: A retrospective study was performed on 16 cases based on the historical diaganosis of cutaneous perineurioma. 10 were located on the extremities (5 upper, 5 lower), 5 on the head/neck, and one on the trunk. The median age of patients was 44 years (range 16-82 years). In addition, 10 cutaneous and soft tissue neurofibromas were used as controls. The following immunohistochemical stains were performed on all 26 cases to characterize the lesions: EMA, CD34, S100, laminin, and collagen IV.

Results: Of the 16 perineurioma cases, 25% were S100 positive, 50% were EMA positive, 93% were CD34 positive in a whorled pattern, 87% were laminin positive, and 81% were collagen IV positive. Most of the cases which were EMA, laminin, and collagen IV positive showed only weak and focal staining. Of the 10 neurofibroma cases, 100% were S100 positive, 0% were EMA positive, 80% were CD34 positive, 100% were laminin positive, and 100% were collagen IV positive. Microscopically, perineuriomas are relatively well circumscribed and consist of a proliferation of delicate, small slender spindled cells, which lack atypia, embedded in a matrix that varies from myxoid to fibrous. There are often foci in which cells are arrayed as loose nests or "whorls" with surrounding myxoid stroma. Neurofibromas are relatively well circumscribed and consist of a proliferation of delicate, wavy spindle cells set in a fibrillar matrix. The clinical, histological, and immunohistochemical findings are summarized in tables 1, 2, and 3 respectively.

Conclusions: When EMA is negative and suspicion of perineurioma is high, CD34 immunopositivity in a whorled pattern is useful in establishing a diagnosis. In addition, weak laminin and collagen IV staining favors a diagnosis of perineurioma over neurofibroma, which typically shows avid immunolabeling with basement membrane determinants. Finally, a positive S100 stain should not exclude the diagnosis of perineurioma if EMA is positive.

387 Membranous CD31 Expression in Juvenile Xanthogranuloma

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Background: Juvenile xanthogranuloma (JXG) remains one of the diseases lacking a well-defined cell of origin. Though once thought to be of dermal dendrocyte etiology because of the proponderence of Factor XIIIa expression, this proposal can not be substantiated fully. More recently, plasmacytoid monocytes have been suggested as the cell of origin and supported by immunohistochemical staining that characterizes cells of this lineage: CD4 and CD45. This pattern further negates the dermal dendrocytic hypothesis and substantiates a hematopoietic progenitor cell etiology. Moreover, it has been shown that endothelial progenitor cells can arise from circulating monocytes in the proper environment. CD31, a transmembrane glycoprotein, that is characteristically used as a marker for vascular endothelium, has recently been shown to be expressed in one case of JXG. To further support the hypothesis that JXGs arise from plasmacytoid monocytic cells, and that these cells are able to differentiate to express CD31, the rate of CD31 expression in JXGs from our archives was investigated.

Design: Twelve cases of JXG, from 8 patients, were retrieved from our files. Four represented pediatric lesions (age 6 months to 9 years) and the remaining 8 were from adults (age 22 to 73 years). The locations represented trunk (n=7), lower extremity (n=3) and head and neck (n=2). The diagnosis was confirmed by review of the available slides. Sections were prepared from the paraffin embedded tissue and stained with antibody to CD31 using standard procedures. CD31 expression was recorded for all cases.

Results: Almost all cases showed XG lesions possessing a mixed population of polygonal and spindled cells and variable number of multinucleated histiocytes referred to as "Touton" giant cells. Only one case consisted of predominately spindle cells, lacking Touton giant cells. CD31 expression was seen in 100% of the cases: focally in 3 and diffuse in the remaining 9.

Conclusions: As hypothesized, all cases express CD31 further supporting the hypothesis of a monocytoid etiology. With this data, further exploration, with a larger series, into the plasmocytoid monocytic pathogenesis of JXG is justified.

388 Is Sentinel Lymph Node Biopsy Indicated in Patients with Desmoplastic Melanoma? A Clinical and Histopathological Evaluation of Pure and Combined Subtypes

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Background: Desmoplastic melanoma (DM) is an uncommon variant of melanoma characterized by prominent stromal fibroplasia. The clinical behavior of DM has been reported to be more similar to sarcomas than conventional melanomas, with limited tendency for lymph node metastasis. Recent data has shown a better long-term prognosis for pure forms of DM as compared to DM combined with other growth patterns. We examined clinical and histopathological features in a series of patients with pure and combined forms of DM, with emphasis on the relevance of sentinel lymph node (SLN) biopsy in patients with DM.

Design: Demographic and staging data from thirty-two patients with DM was retrospectively reviewed. Glass slides of tumors were reviewed in all cases. Tumors were classified as pure (>90%) DM, combined (50-90%) DM, and combined (<50%) DM. Additional microscopic parameters evaluated included presence of an in-situ component, nerve involvement, mitoses/10 high-power fields (10 hpf), and ulceration.

Results: Patient ages ranged from 39-80 (mean 61.6 years) and 75% were male. 59% of tumors were located on the head and neck, followed by the trunk (22%) and extremities (19%). Breslow thickness ranged from 0.85 to 15.0 mm; 25/32 (78%) tumors were >2.0 mm, and 12/32 (38%) were >4.0 mm. All tumors were Clark's level IV (47%) or V (53%). An in-situ component was present in 56% of tumors and ulceration was present in 6%. Pure DM accounted for 12/32 (38%) tumors, 9/32 (28%) were combined (50-90%) DM and 11/32 (34%) were combined (<50%) DM. Nerve involvement was identified in 53% of tumors. No mitoses were present in 44% of tumors, 1-2/10 hpf in 41% and >4/10 hpf in 16%. Sentinel lymph node (SLN) biopsies were performed in 84% of cases, representing 75 lymph nodes. All 75 SLN were negative for metastasis.

Conclusions: This study illustrates the morphologic diversity in tumors diagnosed as DM, as combined DM accounted for the majority (62%) of tumors in our study population. Based on the results of our study, both pure and combined DM demonstrate a lack of proclivity for SLN metastasis. In view of these findings, treatment decisions involving SLN biopsy may require reassessment in patients with both pure and combined subtypes of DM.

389 Detection of Clonal T Cell Receptor Beta Rearrangement Via PCR Based Genescan Analysis in 79 Cases of Cutaneous T Cell-Dominant Infiltrates

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Background: Differentiation of cutaneous lymphoid infiltrates on histologic grounds can often pose a diagnostic challenge for the pathologist. While some unequivocally benign infiltrates are easy to distinguish from cutaneous T cell lymphoma (CTCL), drug-associated lymphomatoid hypersensitivity reaction and cutaneous lesions of collagen vascular disease can demonstrate cytologic atypia, clonality and an immunophenotypic profile that closely simulates CTCL and cause diagnostics difficulties. Similar immunophenotypic and molecular abnormalities to those of malignant lymphoma can also be observed in premalignant lymphoid dyscrasias, such as pityriasis lichenoides chronica (PLC), large plaque parapsoriasis (LPP), atypical pigmentary purpura (PP) and atypical lymphocytic lobular panniculitis.

Design: The purpose of our study was to evaluate a molecular diagnostic test that may aid in the distinction of these various subcategories of cutaneous T cell lymphocytic infiltration. Seventy-nine skin biopsies containing a T cell-rich lymphoid infiltrate were analyzed on paraffin-embedded, formalin-fixed tissue by a novel TCRβ multiplex assay technique.

Results: Our findings indicate that monoclonality and identical molecular profiles between biopsies are characteristic of CTCL with polyclonality being very infrequent. However some cases of drug associated lymphomatoid hypersensitivity, pityriasis lichenoides and pigmentary purpura manifested similar molecular profiles to that observed in CTCL including identical dominant T cell clones present in biopsies procured at varying times and sites, suggesting a common pathogenetic link despite a seemingly disparate clinical presentation and course.

Conclusions: Multiplex PCR assay for TCRbeta rearrangement is a very useful diagnostic test for the categorization of cutaneous T cell infiltrates. It is our opinion that the most information is clearly obtained in cases in which multiple biopsies are performed over time. Identical molecular profiles between biopsies defines a T cell dyscrasia however correlation with the clinical history and other aspects of the light microscopic profile is critical for final delineation. Conversely the finding of polyclonality by TCRbeta gene rearrangements appears to be reliable for reactive states in cutaneous T cell infiltrates while oligoclonality tends to be characteristic for reactive lymphocytic infiltrates associated with endogenous and or iatrogenic immune dysregulation.

390 Analysis of Galectin-3 Expression in Melanocytic Lesions by Tissue Array. Possible Involvement in Tumor Progression

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Background: Malignant melanoma is one of most malignant neoplasms in human. Its incidence has significantly increased in the last 20 years, probably related to changing patterns of sun / ultraviolet light exposure. The pathogenesis of melanoma is still mainly unknown. Most authors accept a multi-step process that may include the phases of benign nevi (BN) and dysplastic nevi (DN) as precursor lesions, and then radial and vertical growth phase melanoma (MM), and metastatic melanoma (MMM). Dysregulation of cellular proliferation and apoptosis is probably involved in melanoma progression and response to therapy. In this study we have studied the expression of galectin-3, a β-galactoside-binding protein involved in apoptosis and T-cell survival, in a large series of melanocytic lesions.

Design: Tissue microarray blocks of 94 melanocytic lesions were constructed and semi-quantitatively evaluated by immunohistochemistry for the cytoplasmic or nuclear expression of galectin-3. For a lesion to be considered positive, it had to show more than 25% of cells with at least mild intensity of labeling. Data were analyzed with the McNemar, Stuart-Maxwell or Fisher-exact tests.

Results: Primary and metastatic melanomas expressed galectin-3 at a significantly higher level than nevi, in both the cytoplasm and the nuclei ($p<0.0073$). For all subgroups, there was a correlation between the levels of nuclear and cytoplasmic expression.

Conclusions: Our results support the notion that melanocytes may acquire higher levels of galectin-3 with tumor progression. Additionally, since galectin-3 expression appears to provide resistance to chemotherapy and immunity, anti-galectin-3 targeted therapy may prove useful in the treatment of patients with malignant melanoma.

391 Histologic Quantification of Tumor Size in Sentinel Lymph Node Metastases Correlates with Prognosis in Patients with Cutaneous Malignant Melanoma

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Background: The three most important prognostic criteria in patients with melanoma are Breslow thickness, tumor ulceration, and sentinel lymph node (SLN) status. Regarding SLN status, breast carcinoma patients are staged depending if the tumor deposit in the node is larger or smaller than 2 mm. It is unknown if a similar cut-off can be used in melanoma.

Design: 1,417 patients underwent SLN examination between 1990 and 2002. All nodes were breadloafed and examined with routine hematoxylin and eosin. Negative cases were examined with immunohistochemistry. In the positive nodes, tumor deposits were analyzed as: number of positive nodes, area occupied by the tumor deposits, length of the largest tumor deposit, number of deposits, location of the deposits (subcapsular vs intraparenchymal), and presence or absence of extracapsular extension. Results were correlated with presence of additional positive lymph nodes in the completion lymphadenectomy and recurrence data (median follow-up of 4.75 years).

Results: Median tumor burden was 1.4 mm^2 for all patients. 69 patients (36.7%) recurred (53 of 58 patients developed stage IV disease). Overall, SLN-positive patients who developed stage IV disease had a significantly greater median SLN tumor burden (5.6 mm^2) compared to SLN-positive patients who did not recur (0.83 mm^2, $p<0.01$). Of 19 patients rendered NED, 7(37%) remained without disease after a median follow-up of 10.5 months; the median tumor burden in this subgroup was 2 mm^2 ($p<0.0001$). Also significantly associated with worse prognosis were the presence of extracapsular extension and intraparenchymatous location ($p<0.0001$).

Conclusions: Microscopic measurement of melanoma deposits and intraparenchymatous location in SLNs strongly correlate with additional positive lymph nodes and disease specific survival. Furthermore, the combination of ulcerated primary melanoma, 3 or more positive SLN, and tumor burden greater than 2 mm^2 strongly predicts poor prognosis. Therefore we recommend that the Pathology report for SLN include the number of positive nodes, length of the largest tumor deposit, location of the tumor deposits, and possible extracapsular extension.

392 Comparison of Chronic Urticaria Patients Responsive and Unresponsive to Antihistamines

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Background: Chronic urticaria (CU) remains a major management problem and antihistamines are a treatment mainstay. Biopsy is rarely performed and few studies have examined the correlation between response to treatment with histologic findings.

Design: Punch skin biopsy of thirty patients evaluated for CU (hives > six weeks duration) over a 3 year period were collected. The histologic reaction pattern and composition of the inflammatory infiltrate was evaluated. These findings were correlated with clinical data.

Results: By histologic inflammatory reaction pattern, suspected CU patients were classified into 2 groups: 1) those exhibiting an urticarial hypersensitivity reaction (UHR) (18/30, 60%) and 2) those with another reaction pattern. In this latter group, only 2/12 patients had symptoms responsive to antihistamines, both of whom showed a dermal hypersensitivity reaction with eosinophils. None of the other 10 patients were antihistamine responsive; the reaction patterns identified in this group was vasculitis (3/12), erythema multiforme-like interface dermatitis (5) and palisaded neutrophilic and granulomatous dermatitis (2). The UHR group could be sub-classified into either those with a neutrophilic (12/18, 67%) or eosinophilic (6/18, 33%) predominate inflammatory infiltrate. All 6 patients with eosinophilic predominate UHR responded to antihistamine therapy (ceterizine, up to 10mg bid). Biopsy of these patients revealed sparse perivascular and interstitial mostly eosinophilic (mean 80 eosinophils versus 0 neutrophils /5 HPF) and lymphocyte rich infiltrates without any evidence of vasculitis. In contrast, all patients with neutrophil predominate (12/18) UHR required additional immunomodulating agents to control symptoms. Biopsies of these lesions showed mild to moderately dense perivascular and interstitial neutrophilic infiltrates (mean 50 neutrophils versus 11 eosinophils /5 HPF). Six of 12 of these neutrophilic predominate UHR also demonstrated rare nuclear debris suggestive of leukocytoclastic vasculitis. By direct immunofluorescence, 3/4 of both eosinophilic and neutrophilic predominate UHR had vascular immunoreactants.

Conclusions: We recommend skin biopsy to be included in the work up of suspected CU as a minority of this clinical population will have another disorder. For patients with true CU (UHR), the biopsy report should state whether the infiltrate is either eosinophilic or neutrophilic predominate. The latter shows overlap with urticarial vasculitis and has symptoms that do not respond to antihistamine therapy.

393 Fli-1 Expression in Cutaneous Mycosis Fungoides

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Background: The Fli-1 nuclear transcription factor is known to play a role in cellular proliferation and tumorigenesis. Expression of Fli-1 has been described in dermal fibroblasts, lymphocytes, endothelial cells, and vascular neoplasms. Evidence for the role of Fli-1 in various neoplasms, and its proven presence in lymphocytes points toward a possible link between Fli-1 dysregulation and the pathogenesis of mycosis fungoides. In this novel study we will further elucidate the possible link between Fli-1 expression and the malignant lymphocytes found in mycosis fungoides.

Design: Sections from formalin-fixed, paraffin-embedded archived specimens were stained using a purified rabbit polyclonal antibody against the highly conserved Fli-1 carboxy terminus. Fli-1 nuclear staining was quantified by light microscopy and stratified based on the percentage of nuclei that showed Fli-1expression. Reactive lymphoid hyperplasia specimens were used as positive controls.

Results: Our data shows that all of the tumor stage lesions show high levels of nuclear Fli-1 expression, paralleling that seen in the reactive lymphocytes of the benign controls. Of plaque stage lesions 6/12 (50%) showed the same intensity, while the remaining 6/12 varied significantly in their FLi-1 nuclear expression. The few patch stage lesions also showed varied expression as shown in Table 1.

Nuclear Fli-1 expression in lymphocytes

	Percentage of nuclei expressing Fli-1				
Diagnosis	0-25	26-50	51-75	76-90	>90
patch stage MF (n=3)	1	1	0	0	1
Plaque stage MF (n=12)	0	3	2	1	6
tumor stage MF (n=4)	0	0	0	0	4
lymphoid hyperplasia (n=4)	0	0	0	0	4

Conclusions: Pathogenesis of MF is not well characterized. This study shows diffuse nuclear expression of Fli-1 in all tumor stage MF, whereas expression of this transcription factor varied widely in the early, epidermotropic stages. Although the numbers are too small to draw statistical significance, this study demonstrates that there is an association between increased expression of Fli-1 and progression to tumor stage MF that merits further investigation. Additionally, the mixed expression of Fli-1 in the epidermotropic stages suggests that the role of Fli-1 in MF is related to neoplasia, not epidermotropism.

394 Psoriasiform Keratosis

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Background: The recognition of lichenoid keratosis as a solitary lesion with a tumor-like clinical presentation is now widely accepted as a distinct clinicopathological condition. The concept of unilesional presentations of conditions that, classically, are widespread is also used, for example, in unilesional expressions of mycosis fungoides. Presented here are 18 cases of erythematous, scaly papules or plaques with microscopic features of both seborrheic keratosis and psoriasis. There was, however, no known clinical diagnosis of psoriasis in any patient, neither at initial presentation nor on follow-up examination.

Design: The cases were collected prospectively from our daily dermatopathology practice and PAS stains were performed on all cases. Patient demographics, lesional characteristics, differential diagnoses, lesion duration, and the possibility of disseminated psoriasis were gathered from the submitted specimens and from the treating physicians.

Results: Most lesions were solitary, present for 6-7 months, and identified on the upper or lower extremities. Other sites included the scalp, neck, shoulders, and back. Men were slightly more affected than women with a mean age of 66.8 years. The most common diagnosis, clinically, was seborrheic keratosis, followed by basal cell carcinoma, Bowen's disease, actinic keratosis, and squamous cell carcinoma among others. The lesions averaged less than a centimeter and were dome-shaped, scaly, and yellow to gray-tan. Histological examination revealed irregular verrucous epidermal acanthosis with hyperkeratosis, parakeratosis, hypergranulosis, and intracorneal collections of neutrophils. Vascular dilatation and lymphocytic chronic inflammation were present in the superficial dermis. PAS stain for yeasts or dermatophytes was negative in all cases. There was no clinical evidence of disseminated psoriasis in any patient, with a mean follow-up of 22.6 months.

Conclusions: We have coined the term "psoriasiform keratosis" as a provisional appellation until the pathogenesis is determined more definitively. It is unclear whether psoriasiform keratoses are rudimentary manifestations of psoriasis, or a lesion *sui generis*.

395 Divergent Expression of the Chemotactic Receptor ChemR23 in Systemic and Pulmonary Langerhans Cell Histiocytosis

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Background: Langerhans cell histiocytosis (LCH) includes clonal neoplastic proliferations of CD1a+ Langerhans cells (LC)(ranging from localized to systemic diseases) and pulmonary LCH, a non-clonal reactive process, frequently associated with heavy smoking or lung neoplasms. ChemR23 is a new chemotactic receptor involved in the recruitment of myelomonocytic cells in response to chemerin, a chemotactic proteins that binds with high affinity ChemR23. Functional ChemR23 is expressed by blood monocytes as well as by myeloid and plasmacytoid DC. On the contrary, LC present in both normal and inflamed skin and LC-derived paracortical interdigitating dendritic cells do not express this receptor. Previous work has suggested that chemotactic factors, such as CCL20/CCR6, may play a crucial role in the development of LCH-associated lesions.

Design: The aim of this study was to investigate the expression of ChemR23 in LCH lesions. Tissue expression of ChemR23 was evaluated by immunohistochemistry in skin (1), oral mucosa (3), bone (1) and lymph node (1) obtained from six cases of systemic LCH and bronchiolo-alveolar (BAL) cells from two cases of pulmonary LCH. Using indirect immunohistochemistry and double immunofluorescence we applied monoclonal antibody anti-ChemR23, CD1a, and CD68, as previously reported (Vermi W., et al. 2005; 201:509).

Results: ChemR23 was found expressed by CD1a+ LC in all patients with systemic LCH but not by LC in pulmonary LCH, where it was expressed by CD68+ macrophages. In normal skin, used as control, ChemR23 was only expressed by dermal dendritic cells whereas LC were negative.

Conclusions: The selective expression of ChemR23 by clonal histiocytes in systemic LCH suggests that this receptor might have a role in directing Langerhans cell multiorgan infiltration. The possible role of ChemR23 in the migration of clonal LC is currently under investigation.

396 Immunohistochemical Analysis of Stromal Responses Does Not Distinguish Benign Cutaneous Lymphoid Proliferations from Cutaneous Lymphomas

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Background: Benign and neoplastic lymphoid proliferations can often be difficult to distinguish from one another based only on histologic examination. Ancillary studies, such as immunohistochemistry (IHC) and molecular studies are often used to resolve difficult cases. We chose to evaluate the stromal responses, using IHC, to determine if significant differences could be determined between benign and neoplastic proliferations of lymphocytes in the skin.

Design: Histologic sections of 9 cases of benign lymphoid proliferations (lichen planus – 5; lupus – 4), and 15 neoplastic lymphoid proliferations (mycosis fungoides (MF) – 7, anaplastic large cell lymphoma (ALCL) – 5, lymphomatoid papulosis (LyP) – 3) were reviewed. Blocks were selected and IHC stains were performed using the following antibodies: collagen IV, CD68, CD1a, podoplanin, smooth muscle actin, Factor XIII, and S-100. In addition, a reticulin stain was also performed. Each stain was graded semi-quantitatively, 0-3+.

Results: The gender and average ages (range) are as follows: benign disorders – 1m/8f, 52 years (33-83), neoplastic disorders – 3m/8f, 40 years (8-74). Refer to Table 1 for results. Mean results for each stain were reviewed using a student T-test. No statisitically significant differences were identified.

Conclusions: No statistically significant differences were identified in our immunohistochemical evaluation of stromal repsonses in benign and neoplastic cutaneous lymphoid proliferations. There is considerable individual variation in stromal responses to lymphoid proliferations. Surprisingly strong stromal responses were seen in two cases of MF, both of which were in very young patients. It is possible that review of larger numbers of cases may clarify these results.

Table 1. IHC results

Stain	Benign	Neoplastic
Coll IV	0.78	0.40
Reticulin	1.89	2.13
Podoplanin	0.33	0.20
CD68	1.78	1.93
CD1a	0.44	0.87
SM Actin	0	0
Factor XIII	0.44	0.80
S-100	1.44	1.13
Ki-67	0.56	1.27

397 Fli-1 Expression in Malignant Melanoma

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Background: Friend leukemia integration site 1 (Fli-1) has been reported as the first nuclear marker of endothelial differentiation. This ETS transcription factor in known to be widely expressed in leukocytes and was also recently demonstrated in melanomas. The expression of Fli-1 in melanoma varied from strong to weak or entirely absent in many cases. We have investigated whether Fli-1 is found in melanoma cell lines and whether the variability of Fli-1 expression is associated with progression of the melanoma.

Design: Formalin-fixed, paraffin-embedded tissue sections from 69 primary melanomas and 28 metastatic melanomas, were included. The median age of the patients was 62 years (range 19–93 years). Of the primary tumors, 53 were classified as superficial spreading and 15 as nodular. All tissues were immunostained by Fli-1, Ki-67, and Ets-1 by standard synthetic polymer-based methods. Five melanoma cell lines were evaluated by Western blot and also by immunocytochemistry.

Results: Fli-1 expression had positive correlation with metastases (higher in metastatic tumors) (r=0.208, p=0.041, Spearman correlation) and positive correlation with percent positive cells by Ki-67 (r=0.233, p=0.022, Spearman correlation). Positive correlation with the presence of ulcer in the primary tumor was found (r=0.228, p=0.48, Spearman correlation). There was also positive correlation with Ets-1 expression (r=314, p=0.002, Spearman correlation). In primary tumors, there was no association with depth of invasion, Clark levels or the presence of lymphoid infiltrate. Also, there was no association with patients' age or gender.

Conclusions: Flil-1 expression in melanoma appears associated with some indicators of tumor progression including tumor proliferation fraction as measured by Ki-67 expression, the presence of ulcer in the primary lesion, and higher expression in metastatic tumors.

398 Metastatic Basal Cell Carcinoma Exhibits Reduced Actin and Calponin Expression

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Background: Basal cell carcinoma (BCC) is the most common malignancy in Caucasian individuals. Metastatic BCC is extremely rare with the most recent studies suggesting that the rate is approximately 0.03%. Actin proteins have been detected in aggressive forms of BCC and might be involved in metastatic lesions. We compared the expression of actin and actin-related cytoskeletal proteins in relatively less aggressive BCCs, (nodular), aggressive (infiltrative/morpheaform), and metastatic BCC.

Design: We studied 12 cases of nodular BCC, 10 cases of infiltrative BCC, and 5 cases of metastatic BCC with immunohistochemistry for alpha-smooth muscle actin (SMA), calponin, myosin, and E-cadherin. Expression was scored semiquantitatively as follows: number of cells (0 = less than 5%; 1 = 5-25%; 2 = 26-75%; 3 = >75%) and intensity (0 = none; 1 = weak; 2 = moderate; 3 = intense).

Results: Actin was present in 3/12 (25%) of nodular BCCs, 10/10 of the infiltrative BCCs, and only 1/5 of the metastatic BCCs. Calponin was present in 6/12 of the nodular BCCs, 6/10 of the infiltrative BCCs, and 2/5 of the metastatic BCCS. Myosin was not present in any of the cases. E-cadherin was present in 8/12 of the nodular BCCs, 7/10 of the infiltrative BCCs, and 4/5 of the metastatic BCCs. Therefore, there was a decreased expression of actin and calponin in metastatic versus infiltrative BCCs.

Conclusions: Our results suggest that increased actin and calponin may contribute to local invasiveness, but are lost in the metastatic phenotype. Significant loss of E-cadherin is not a feature of the aggressive or metastatic forms of BCC as expression was uniform in all types.

399 Role of C5b-9 in the Pathogenesis of the Cutaneous Hyalinized Vasculopathies of Diabetes Mellitus and Porphyria Cutanea Tarda

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Background: The cutaneous lesions of diabetes mellitus and porphyria cutanea tarda exhibit similar pathological findings, including vascular hyaline deposition, and thickening of the basement membranes. The effector mechanism of vascular injury has remained elusive. The purpose of this study was to explore the role of C5b-9 as a critical mechanism eventuating in the microvascular injury inherent to both of these disorders. The potential mechanisms leading to complement activation in each is explored.

Design: Skin biopsies from 15 patients with cutaneous manifestations of diabetes mellitus and 10 patients with porphyria cutanea tarda were examined using light microscopy and immunofluorescence for IgG, IgA, IgM, C3, and C5b-9.

Results: The 15 patients with diabetes mellitus ranged in age from 25 to 80 years, mean age of 60. There was an equal distribution of patients with type I and type II DM. Four of these patients had bullosa diabeticorum while the other patients had other cutaneous lesions including nephrogenic fibrosing dermopathy and necrobiosis lipoidica. The 10 patients with PCT ranged in age from 28 to 59 years. All had classic lesions of PCT. In all biopsies, the DIF profiles showed striking deposits of C5b-9 within vessels in a granular array with concomitant homogeneous vascular staining.

Conclusions: C5b-9 is the apparent effector mechanism of vascular injury in the setting of DM and PCT albeit through different mechanisms In PCT the basis of C5b-9 is light induced complement activation of circulating porphryins. In the setting of DM preferential glycation of CD59 leads to its inactivation. The role of C5b-9 in the pathogenesis of these diseases suggests the potential use of biologics for prevention and treatment, namely anti-C5b-9.

400 Expression of Cancer Testis (CT) Antigen NY-ESO-1 in Primary and Metastatic Malignant Melanoma (MM), Correlation with Prognostic Factors and Potential Role in a Melanoma Vaccine

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Background: CT antigens are potential targets for cancer immunotherapy as they are expressed in various cancers but not in normal adult tissues except testis. NY-ESO-1 is among the most immunogenic CT antigens, eliciting humoral and cellular responses. Recent clinical trials using NY-ESO-1 protein/peptides in MM have shown clear evidence of inducing immunity. However, little is known about the differential expression profile of NY-ESO-1 in primary and metastatic MM and its relationship to disease severity. Here, we analyzed the expression of NY-ESO-1 in a series of primary and metastatic MM, and its relation to prognostic factors.

Design: IHC was done with mAb E978 to NY-ESO-1. We studied 60 primary MMs and corresponding sentinel lymph node (SLN) metastases. 15/60 SLNs showed metastatic MM. 14/15 SLNs were available for IHC. 12 additional metastatic MMs from other patients were also included in this study (total of cases was: 60 primary and 26 metastatic MMs).

Results: NY-ESO-1 was expressed in 6/60 (10%) primary MMs and in 8/26 (30%) metastatic MMs. 3/8 positive cases were from SNLs; in 2/3 positive SLNs, the primary was also positive while in 1/3 positive SLNs, the primary was negative. Gender distribution of NY-ESO-1 positive MMs (primary or metastasis) was even. The median patient age was 60 years for NY-ESO-1 positive and negative cases. The median MM primary thickness was 4.3 mm (NY-ESO-1 positive cases) and 2.8 mm (NY-ESO-1 negative cases). Primary lesions were nodular in 83% and ulcerated in 50% of NY-ESO-1 positive cases versus 24% and 22% in NY-ESO-1 negative cases respectively. 3/6 (50%) of the NY-ESO-1 positive primaries were associated with metastases compared to 10/54 (18.5%) NY-ESO-1 negative cases.

Conclusions: NY-ESO-1 is more frequently expressed in metastatic than in primary MM and its expression appears to be associated with thicker primary lesions, nodular growth pattern, ulceration and higher frequency of metastatic disease indicating a marker of worse prognosis. Our study indicates that at least 30% patients with metastatic MM express NY-ESO-1 and hence may be candidates for NY-ESO-1 based immunotherapy.

401 Neutral Endopeptidase (NEP) Overexpression Is Associated with Progression in Malignant Melanoma (MM) and Is a Potential Target of Treatment

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Background: Altered expression (both loss and overexpression) of NEP, a cell surface peptidase, has been reported in several tumors. Investigation into the role of NEP upregulation in tumor progression is clinically relevant given the availability of NEP inhibitors. The aim of this study was to determine the clinical relevance of altered NEP expression in a well-characterized cohort of MM patients and cell lines.

Design: We examined NEP protein expression in paraffin-embedded tissues using an immunohistochemical assay in 72 patients, including 38 primary (thickness <1mm, n=15; 1-4mm, n=18; >4mm, n=5) and 34 metastatic MM. We also assessed NEP transcript expression using Affymetrix U133Plus2.0 GeneChips in 22 metastatic MM cases. In addition, we determined NEP protein expression in 7 MM cell lines using western blotting and silenced it using siRNA in 2 melanoma cell lines (SKMEL-19 and MeWo).

Results: In MM patients' samples, NEP protein overexpression was more significantly observed in metastatic MM compared to primary (19/34 [55.9%; 95% CI=38.1%, 72.4%] versus 1/38 [2.6%; 95% CI=0.1%, 15.4%], respectively, p<0.0001). The only positive primary case was an 11.0 mm thick, Clark level V, ulcerated acral MM. In addition, 8/22 (36.4%; 95% CI=18.0%, 59.2%) metastatic MM cases showed a 3-11 fold increase in NEP transcription. Preliminary analyses of survival data suggest that patients whose metastatic tumors expressed NEP had no worse overall survival compared to those who did not express NEP (p=0.85 by log-rank test), although there was limited statistical power available to detect potential survival differences. In-vitro, NEP was overexpressed in 4/7 (57%) MM cell lines screened, and down-regulation of NEP resulted in down-regulation of the pAkt in 1/2 cell lines tested.

Conclusions: Our data suggest that NEP upregulation is related to tumor progression in MM. More studies are needed to define the role of NEP in melanoma pathogenesis and progression as well as the association between NEP dysregulation and response to NEP inhibitors in MM.

402 Immunohistochemical and Iron Staining Are Useful in Differentiating Kaposi's Sarcoma (KS) from Interstitial Granuloma Annulare (GA)

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Background: KS is a cutaneous and deep tissue neoplasm composed of perivascular/interstitial spindle cells associated with a prominent vascular component in well-developed lesions. Early KS may present a significant diagnostic problem. GA is an idiopathic dermatosis characterized by a dermal infiltrate of histiocytes, fibroblasts, and lymphocytes. In some instances, early KS may be difficult to differentiate from the interstitial form of GA, as both lesions share common features of an increased number of ovoid to spindle cells situated between collagen bundles in the reticular dermis. We characterized specific immunohistochemical (IH) and iron staining profiles to distinguish these two entities with histologic overlap and markedly distinct clinical implications.

Design: 9 "early" and 10 typical KS cases, and 10 interstitial and 10 palisaded GA cases were studied. "Early" KS cases displayed interstitial spindle-shaped and dendritic cells without a well-developed dermal vascular proliferation. Tissue sections were stained for iron, HHV-8, CD31, CD34, collagen IV, Factor VIIIa, and MIB-1.

Results: Iron staining of lesional dermis was confirmed in all cases (19/19) of KS and no cases (0/20) of GA. IH stains for HHV-8 were positive in all (9/9) cases of "early" KS and most (9/10) cases of typical KS. All cases of GA (20/20) were negative for HHV-8. Collagen IV was positive in a subset of KS cases: 4/9 early and 6/10 typical KS; and negative in all 20/20 cases of GA. A subset of GA was positive for CD31, CD34, and Factor VIIIa as follows: CD31 - interstitial GA 6/10, palisaded GA 8/10; CD34 - interstitial GA 9/10, palisaded GA 4/10; Factor VIIIa - interstitial GA 5/10, palisaded GA 3/10. All KS showed positive staining for CD31, CD34 and Factor VIIIa. KS and GA had similar patterns of MIB-1 reactivity.

Conclusions: Iron staining and IH staining for HHV-8 in combination are reliable markers (100% sensitivity and 100% specificity) for the diagnosis of KS when interstitial GA is considered in the differential diagnosis. Collagen IV was detected in KS, but was an insensitive marker for the diagnosis. CD31, CD34, and Factor VIIIa were positive in all KS and in some GA; and the diagnosis of KS is less likely when these markers are negative. This study provides novel data regarding the comparative immunophenotypic and histochemical (iron staining) characterization of subtypes of KS and GA.

403 BRAF and c-kit Copy Number in Mutation Positive Malignant Melanoma

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Background: Activating mutations in several types of tyrosine kinases have recently been reported in a variety of human malignancies. Most reports indicate that the vast majority of these mutations are heterozygous. This would be expected because of the presumed dominance of the mutation. We have recently used the technique of High Resolution Melting Amplicon Analysis (HRMAA) to screen for BRAF and c-kit activating mutations in a series of melanomas. All cases were followed up with direct

DNA sequencing. Inspection of the DNA sequencing electropherograms suggested that not all cases of BRAF or c-kit mutation positive melanoma are heterozgous.
Design: Forty three cases of BRAF mutation positive and two cases of c-kit mutation positive malignant melanoma were described previously (Hum Pathol. 2005 May:36(5):486-493). An additional 50 cases of malignant melanoma to evaluate for c-kit activating mutations were retrieved from the pathology files at University of California, Los Angeles (UCLA) and an additional 3 cases were retrieved from the University of Utah. The additional cases were immunohistochemically stained for c-kit and positive cases screened for c-kit activating mutations by HRMAA. The DNA sequencing electropherograms from all mutation postive melanomas were carefully reviewed in order to estimate the amount of the mutant allele. Cases in which the electropherograms suggested at least 65% of electropherogram peak was composed of the mutant allele were classified as mutant allele increased. Fluorescence in situ Hybridization (FISH) for BRAF and c-kit were performed on cases which appeared to be mutant allele increased.
Results: Of the 43 BRAF mutation positive tumors, 9 (21%) cases appeared to contain an excess of the mutant allele. BRAF FISH on these 9 cases suggested the increased amount of the mutant BRAF allele was due to amplification (2 cases) or chromosome 7 polysomy (7 cases). One of 53 additional cases of melanoma (2%) contained the c-kit activating mutation L576P. Sequencing electropherograms from all three cases c-kit L576P mutation positive melanoma suggests a selective loss of the normal allele. C-kit FISH indicated that one case showed slight amplification of the mutant allele and the other two were probably homozygous.
Conclusions: Some BRAF mutation positive melanomas have an increased copy number of the mutant allele. C-kit activating mutations in melanomas are confined to L576P in exon 11.

404 Decreased Srcasm Expression in Squamous Cell Carcinoma

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Background: The Src-activating and signaling molecule (Srcasm) is a recently described activator and substrate of Src-family tyrosine kinases (SFKs). When phosphorylated, Srcasm binds to important signaling molecules and modulates epidermal growth factor-dependent signals trasmited by SFKs in keratinocytes. In vitro studies have shown that elevated Scrasm levels inhibit keratinocyte proliferation while promoting differentiation. If elevated Srcasm levels promote differentiation, then its expression may be altered during neoplasia to promote growth. The goal of this study is to determine if Srcasm expression is altered in tissue specimens of squamous cell carcinoma when compared to unremarkable epidermis.
Design: Initially, four cases of cutaneous squamous cell carcinoma (SCC) and four corresponding normal skin samples were analyzed for Srcasm gene expression. Unremarkable epidermis and SCC samples were obtained after Moh's surgery and frozen at -80°C until RNA isolation. First-strand cDNA synthesis was followed by quantitative RT-PCR for the human Scrasm gene. Results were compared to a standard curve utilizing a plasmid containing human Srcasm. Change in gene expression was considered as significant when at least a 2- fold variation. In a subset of SCC and unremarkable epidermal samples, Srcasm protein levels were evaluated by western blotting of lysates.
Results: The levels of Scrasm mRNA expression were evaluted in four cutanous SCC and compard to levels of Srcasm expression in normal epidermis in the same patient. The average fold stimulation of Srcasm expression in SCC when compares to normal epidermis was 4.57 fold lower in SCC (SD 3.03). Western blot analysis also showed that Srcasm protein levels in cutanous SCC were lower than in normal skin.
Conclusions: Our study demonstrates that Srcasm gene and protein expression is downregulated in SCC when compared to unremarkable epidermis. As high levels of Srcasm promote differentiation, its decreased expression may by an important means of inhibiting keratinocytes differentiation, thereby increasing cell proliferation.

405 Expression and Cellular Localization of S100A4 Protein in Melanocytic Lesions

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Background: S100A4 is a member of the S100 family of calcium-binding proteins. The expression of S100A4 is increased in melanomas and breast carcinomas *in vitro* and *in vivo*. Higher expression has also been correlated with poor prognoses for patients with carcinomas of other organs. S100A4 affects multiple aspects of tumor behavior including motility, invasion, and apoptosis (Helfman DM *et al*, Br J Cancer 2005;92:1956). The aim of this study is to compare the expression and localization of S100A4 protein in not only melanocytic tumors but also benign nevi.
Design: 5-micron sections of tissue microarrays containing normal tissue and 65 melanocytic lesions (19 benign nevi, 17 in-situ and invasive melanomas, and 29 metastatic melanomas) were mounted on covalent slides. Monoclonal antibodies for S100A4 were assayed using standard ABC immunohistochemical techniques with a red alkaline phosphatase substrate. Slides were reviewed by two pathologists and lesions were scored as negative or positive if >10% of cells were stained. Positive staining was further classified as cytoplasmic, nuclear, or both. The chi-square test was used to evaluate group comparisons.
Results: Expression of S100A4 in different melanocytic lesions is summarized below:

	Benign Nevus	Primary Melanoma	Metastatic Melanoma	Total
S100A4 Negative	14	8	9	31
S100A4 Positive	5	9	20	34
Total	19	17	29	65

The majority of benign nevi (74%) did not express S100A4. A stepwise increase in the proportion of lesions expressing S100A4 was observed from nevi (5/19) to primary melanomas (9/17) to metastatic melanomas (20/29) (p=0.02). Of the lesions that expressed S100A4, expression limited only to the cytoplasm was not seen in nevi (0/5) and occurred only in primary melanomas (8/9) and metastatic melanomas (11/20) (p=0.01). Nuclear as well as cytoplasmic expression was seen in all nevi (5/5), a minority of primary melanomas (1/9), and some metastatic melanomas (7/20).
Conclusions: Increased S100A4 protein expression is acquired by melanocytic cells in the progression from benign nevic precursors to primary melanomas to metastatic tumors. S100A4 protein localized to the cytoplasm is associated with a malignant phenotype. These results suggest that S100A4 upregulation in tumor progression reflects previously demonstrated aggressiveness in malignancies. In addition, S100A4 staining may be considered as a diagnostic adjunct in differentiating nevi and melanomas.

406 Expression of D2-40 in Eccrine Spiradenoma

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Background: Eccrine spiradenoma has been known as a skin adnexal neoplasm with eccrine differentiation. Histologically, eccrine spiradenoma characteristically displays two types of cells: the centrally located epithelial cells with pale staining nuclei and, the periphery located smaller cells with dark nuclei. The former are arranged around a small ductal lumina. Like some neoplasms of vascular origin, spiradenoma usually present as a painful skin nodule. In this study, we investigate the expression of CD2-40, a specific lymphatic marker and CD31 and CD34 in skin adenaxal neoplasm and epidermal neoplasm.
Design: A total of 55 cases including 10 eccrine spiradenomas, 10 eccrine poromas, 10 eccrine hidradenomas, 5 trichoepitheliomas, 10 basal cell carcinoma, and 10 squamous cell carcinoma, were retrieved from the hospital computer system. Immunostaining for D2-40, CD31 and CD34 were performed on an automated immunostainer with appropriate positive and negative controls. The statistical analysis was performed with Chi-Square test.
Results: All 10 cases of eccrine spiadenoma show strong and diffuse immunreactivity with D2-40. No immunoreactivity was observed in any of the 45 cases of eccrine poroma, eccrine hidradenoma, trichoepithelioma, basal cell carcinoma and squamous cell carcinoma. All 55 cases of eccrine spiradenoma, eccrine poroma, eccrine hidradenoma, trichoepithelioma, basal cell carcinoma and squamous cell carcinoma were negative for CD31 and CD34.
Conclusions: Our results indicate that D2-40 is a very sensitive and specific markers for eccrine spiradenoma. In addition, eccrine spiradenoma may not be an eccrine neoplasm since D2-40 is a specific marker of lymphatic channel, is not found in other eccrine skin adnexal neoplasms.

407 Immunohistochemical Expression of Microtubule Associated Protein-2 (MAP-2) in Eccrine Poroma

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Background: Microtubule-associated protein-2 (MAP-2) is a cytoskeleton protein associated with microtubule assembly, cell proliferation and growth. In our previous study of MAP-2 in skin neuroendocrine neoplasm, we observed that MAP-2 is selectively expressed in normal eccine glands. Therefore, we hypothesize that MAP-2 may be a useful marker for neoplasm with eccrine differentiation. In this study, we investigate the diagnostic utility of MAP-2 in skin adenexal neoplasm as well as epidermal neoplasms.
Design: A total of 55 cases including 10 eccrine poromas, 10 eccrine spiradenomas, 10 eccrine hidradenomas, 5 trichoepitheliomas, 10 basal cell carcinoma, and 10 squamous cell carcinoma, were retrieved from the hospital computer system. Immunostaining for MAP-2 was performed on an automated immunostainer with appropriate positive and negative controls. The statistical analysis was preformed with Chi-Square test.
Results: 9 of 10 cases of eccrine poroma (90%) show strong and diffuse immunreactivity with MAP-2. No immunoreactivity was observed in any of the 35 cases of eccrine spiradenoma, eccrine hidradenoma, trichoepithelioma, basal cell carcinoma and squamous cell carcinoma.
Conclusions: MAP-2 is a useful marker in separating eccrine poroma from other skin adenexal and epidermal neoplasms, since MAP-2 is expressed in eccrine poroma, but not in other eccine neoplasms.

408 Cluster Analysis of Small Tissue Microarray Dataset of Key Differentiation and Progression Antigens in Melanoma Identifies Groups with Differential SMAC Levels

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Background: A variety of molecules have been associated with melanoma differentiation or progression. It is not known whether these antigens are expressed in biologically meaningful patterns. It is difficult to appreciate such patterns by conventional clinicopathological and statistical methods, as these methods make assumptions of classification *prior* to analysis. Unsupervised hierarchical analysis is a major tool for data mining and is used widely in large gene expression dataset analysis. Recently, it has also been applied to tissue microarray data, mainly for correlating immunomarker profiles with clinical variables, particularly outcomes. In this study, we explored the usefulness of hierarchical cluster analysis for small tissue microarray dataset.
Design: Tissue microarrays of forty-nine melanoma samples were constructed by the technique described by Chen and Zhou (*Am J Clin Pathol*, 124:103) and labeled by standard immunohistochemistry procedures. Expression patterns of five key melanoma differentiation (HMB45, MART1 and S100) and progression (BIRC5, MIB1) antigens

were analyzed by unsupervised hierarchical cluster analysis. The clustering results were further tested statistically and for expression of proapoptotic molecules.
Results: (1) The cases were clustered into four categories, each with a characteristic expression pattern of the antigens. (2) The clusters were further analyzed by pairwise nonparametric test for their differences in antigen expression. Significance analysis of microarray indicated that MART1, MIB1 and BIRC5 were significant in separating the clusters, while the other antigens and clinical variables were not. (3) Importantly, on further testing of expression of key pro-apoptotic molecules, the clusters differed in expression of the mitochondrial protein SMAC/Diablo, an antagonist for the inhibitor of apoptosis proteins.
Conclusions: Hierarchical clustering analysis, even of small tissue microarray dataset, could help reveal hidden patterns not recognizable by conventional clinicopathological and statistical analysis. Clustering generates models testable for their biological significance. We emphasize the exploratory and hypothesis-generating function of clustering.

Endocrine

409 ISL1 Expression in Human Pancreas and in Pancreatic Endocrine Tumors

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Background: The LIM homeodomain protein Isl1 is expressed in developing pancreas in rodents. It is essential for the generation of pancreatic endocrine cells: targeted disruption of the *isl1* gene results in an early arrest of embryonic development with abnormality of the pancreas anlage and complete absence of endocrine cells. Isl1 expression in normal and neoplastic human pancreas has not yet been evaluated.
Design: To evaluate Isl1 protein and mRNA expression in developing and adult human pancreas and in pancreatic and extrapancreatic endocrine tumors. Methods. Normal samples of fetal (4) , pediatric (3) and adult (10), paraffin embedded pancreatic tissues were evaluated for Isl1 expression with immunohistochemistry utilizing two different monoclonal antibodies. A large series of pancreatic endocrine tumors -PET-, including benign (50), borderline (29), well differentiated (30) and poorly differentiated carcinomas (10), pancreatic ductal adenocarcinomas (20), lung (30) and gastrointestinal (60) endocrine tumors were immunostained. Selected samples of normal (4) and neoplastic cases (10) were evaluated for Isl1 mRNA expression with a RT-PCR technique on frozen material.
Results: Isl1 was expressed in the nuclei of pancreatic endocrine cells from the beginning of endocrine differentiation in the fetus till in the adult life. Isl1 reactivity was confined to the endocrine pancreatic compartment. All insulin and glucagon positive cells coexpressed Isl1. Both antibodies gave the same reaction pattern. No Isl1 immunoreactivity was observed in ductal adenocarcinomas. Nuclear Isl1 immunoreactivity was present in 87% of PET cases. Poorly differentiated pancreatic endocrine carcinomas displayed a decrease or absence of Isl1 immunoreactivity; no correlation with prognosis was observed. Ileal carcinoid cases were negative for Isl1, as well as most other low grade pulmonary and GI tract endocrine tumors; intense Isl1 staining was present in lung and GI small cell carcinomas. RT-PCR analysis paralleled ICH results.
Conclusions: Isl1 is expressed in developing and adult human endocrine pancreas, at variance with rodent pancreas where its expression is limited to the embryonic life. Isl1 expression is preserved in most pancreatic endocrine tumors but is absent in most lung and GI tract endocrine tumors, with the exception of small cell carcinomas, with potential diagnostic applications.

410 Precursor Lesions in Patients with Multiple Endocrine Neoplasia Type 1-Associated Duodenal Gastrinomas

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Background: The identification of precursor lesions has a great impact on the understanding of tumorigenesis. Precursor lesions of endocrine tumors are known to occur in the setting of the MEN1 syndrome. It was the aim of our study to test the hypothesis that MEN1-associated duodenal gastrinomas originate from diffuse preneoplastic gastrin cell changes. Precursor lesions may precede the development of duodenal gastrinomas, since, in contrast to sporadic gastrinomas, these tumors are usually multiple.
Design: The distribution of endocrine cells in the nontumorous duodenal tissue was analyzed qualitatively and quantitatively in 25 patients operated on for a duodenal gastrinoma. The MEN1 status was assessed clinically and by PCR based mutational analysis.
Results: Fourteen of 25 gastrinoma patients had proliferative, hyperlastic lesions consisting of gastrin cells in the nontumorous duodenal mucosa similar to the gastric ECL cell lesions observed in chronic atrophic gastritis. All ZES patients with proven MEN1 had such proliferative gastrin cell lesions, and all ZES patients without precursor lesions were MEN1 negative.
Conclusions: Duodenal gastrinomas in MEN1, but not sporadic duodenal gastrinomas, are associated with proliferative gastrin cell changes within the nontumorous mucosa. It is likely that these lesions precede the development of MEN1-associated duodenal gastrinomas.

411 Differential Topographic Kinetics in the Progression of Follicular Thyroid Neoplasms

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Background: The kinetic differences (proliferation and apoptosis) and their correlation with telomere length and telomerase expression by topographic compartments have not been studied in follicular thyroid proliferative lesions to date.
Design: We selected 82 follicular thyroid proliferative lesions (9 hyperplastic nodules, 22 adenomas, 14 minimally-invasive carcinomas, 24 widely-invasive carcinomas and 13 anaplastic carcinomas, classified according to WHO criteria). Representative samples were evaluated and selected for Ki-67 and telomerase immunostaining, In situ end labeling (ISEL) of DNA fragments (to detect apoptosis using Klenow fragment of DNA polymerase), and FISH-PNA of telomere in peripheral and internal tumor compartments. Appropriate controls were run in each sample. The results were statistically compared using analysis of variance and Student t-test, and considered significant if $P<0.05$.
Results: The Ki-67/ISEL indices revealed the predominant kinetic advantage in the internal compartments of benign follicular thyroid proliferative lesions and in the peripheral compartments of malignant follicular thyroid proliferative lesions due to statistically significant decrease of ISEL indices at internal compartment of benign follicular thyroid proliferative lesions (5.3±7.8% vs. 1.3±2.4%; P=0.0213) and at peripheral compartments in carcinomas (1.4±1.7% vs. 2.3±5.5%; P=0.0474), respectively. Telomerase expression was significantly higher in the internal compartments ($p<0.001$) and in malignant lesions ($p<0.001$), which only correlated with PNA-FISH detectable telomeres in internal compartments. PNA-FISH detectable telomeres in more than 20% of peripheral tumor cells was observed in high-grade lesions (widely-invasive and anaplastic carcinomas) only.
Conclusions: 1. Inverse and opposite proliferation/apoptosis correlations characterized follicular thyroid proliferative lesions, the kinetic advantage predominating in the internal compartment of benign lesions and in the peripheral ones of malignant lesions. 2. The direct telomere-telomerase correlation characterizes expansive internal tumor compartments, while the telomere preservation (even in the absence of detectable telomerase expression) at peripheral compartments kinetically defines high-grade lesions.

412 Adrenal Myelolipomas Show Non-Random X-Chromosome Inactivation in Hematopoietic Elements and Fat: Support for a Clonal Origin of Myelolipomas

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Background: The question of whether or not the hematopoietic cells of myelolipoma are truly 'normal' has not been evaluated extensively. In at least one study, a clonal cytogenetic abnormality was identified in a myelolipoma. We examined histologic, immunohistochemical features and comparisons of X-chromosome inactivation patterns in 19 myelolipomas.
Design: Formalin-fixed, paraffin-embedded archival tissue from 19 myelolipomas (16 adrenal; 3 – presacral) was stained with H&E and for CD138, CD34, CD117, CD42a, Hgb, myeloperoxidase, collagen IV, nerve growth factor receptor and reticulin. Histologic evaluation included: overall cellularity of hematopoietic tissue, myeloid to erythroid ratio, and numbers of megakaryocytes. X-chromosome inactivation analysis was performed on hematopoietic tissue, fat and adjacent adrenal tissue (normal control) from 11 female patients by PCR.
Results: Myelolipomas showed wide variation in cellularity (5% to 90%) with no relationship between cellularity and the patient's age. All of the myelolipomas demonstrated normal trilineage hematopoiesis and cellular morphology, with only rare early myeloid precursors were shown to be present in these lesions (CD117, CD34 staining). Most (14/19) had numerous megakaryocytes. The majority had a stromal composition and vascular patterns that were different from those of normal bone marrow. 18/19 cases showed reticulin fibrosis, 14/19 cases showed an increased nerve growth factor receptor and reticulin staining, suggesting increased stromal elements. X-chromosome inactivation studies demonstrated non-random X-chromosome inactivation in 8/11 myelolipomas from female patients, suggesting that both the fat and hematopoietic elements are a single, clonal population.
Conclusions: Non-random X-chromosome inactivation in fat and hematopoietic cells suggests that myelolipomas are clonal proliferations rather than hamartomas. These lesions have significant morphologic and stromal differences from the normal bone marrow.

413 Expression of Notch3 Protein in "High Altitude Paragangliomas"

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Background: Notch proteins are transmembrane receptors that participate in cell fate decisions during development and may play a role in differentiaton of neuroblastomas and other tumors. In a previous pilot study of genetically characterized pheochromocytomas (PC) and extra-adrenal paragangliomas (PGL) we found diffuse Notch3 immunoreactivity in 2 apparently sporadic adrenal PC and in 1 of 2 extra-adrenal PGL with a succinate dehydrogenase (SDH) mutation. PC occurring in von Hippel-Lindau (VHL) disease showed focal staining, while other syndromic tumors were negative. Both VHL and SDH tumors express markers of hypoxic signaling. The previous findings therefore suggested an association of Notch3 with an uncharacterized subset of PC/PGL and a potential association with hypoxia.