tumor (PEN). This study presents our experience with this tumor with emphasis on two morphologic variants: SPT with signet ring cells, and SPT with clear cells.

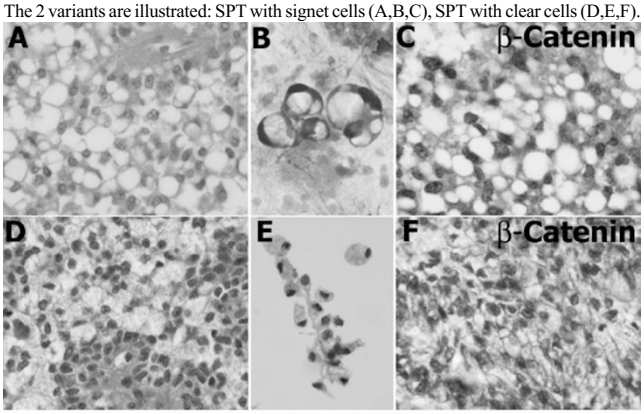
Design: Fifteen histologically confirmed SPT were identified in our files. Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) was performed in 8/15 cases. Patients' demographics, cytohistologic correlation and tumor characteristics were evaluated.

Results: The results are summarized in the table.

Age (yr)	Sex	Site	Size (cm)	FNA Dx	Gross
23	F	Body/tail	1.7	Nondiagnostic	Solid
36	F	Body/tail	2.3	SPT vs. PEN	Solid
27	M	Body/tail	3.6	Malignant with signet ring features	Solid
36	F	Body/tail	3.5	SPT	Solid
55	F	Body/tail	3.5	SPT vs. PEN	Cystic
50	F	Body/tail	8.5	SPT	Solid & cystic
39	F	Head	1.5	SPT	Solid
25	F	Body/tail	2	SPT with vacuolated cells	Solid & cystic
17	F	Head	3	ND	Solid
45	M	Body/tail	NA	ND	Solid
23	F	Head	6.5	ND	Solid
24	F	Body/tail	8.5	ND	Cystic
42	F	Body/tail	NA	ND	NA
73	M	Body/tail	3.2	ND	Solid
38	M	Body/tail	2.7	ND	Solid

ND - not done; NA - not available

11/15 subjects were female and 4 were male with an age range of 17-73 years. 12 SPT were located in the pancreatic body/tail, and 3 in the head. Tumor size ranged from 1.5-8.5 cm and 10 were solid. Of the 8 EUS-FNA, 4 were diagnosed as SPT, 2 as SPT vs. PEN, 1 as malignant with signet ring features, and 1 was nondiagnostic. Immunohistochemistry (IHC) was performed on cell blocks in 6/8 FNAs. Panels included β-catenin, CD10, vimentin, CD56, synaptophysin, chromogranin and keratin. The 2 variants are illustrated: SPT with signet cells (A,B,C), SPT with clear cells (D,E,F).



A, B, C - SPT with signet ring cell features. D, E, F - SPT with vacuolated cytoplasm.

Conclusions: SPT may occur in males and older adults, and present as a small or solid tumor. Variants with signet cells or clear cytoplasm may pose a diagnostic challenge. However, when combined with the appropriate IHC studies, an accurate diagnosis can be provided by FNA and histologically. Awareness of the wide spectrum of its clinical presentations and morphologic variants can prevent diagnostic pitfalls.

460 Should LSIL-H Be a Distinct Cytology Category? A Study on Frequency and Distribution of 40 HPV Genotypes in a Cohort of Underserved Women

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Background: The Bethesda System (TBS 2001) for gynecologic cervical cytology reporting classifies squamous intraepithelial lesions (SIL) into low grade (LSIL) and high grade (HSIL) lesions. In clinical practice, an intermediate term LSIL-H has been used in a small percentage of LSIL cases with atypical squamous cells insufficient qualitatively or quantitatively to diagnose HSIL. However, the diagnostic criteria of LSIL-H are not defined and little is known about HPV status in those patients. We therefore analyzed the frequency and distribution of 40 HPV genotypes among expanded cytology categories including LSIL-H.

Design: A total of 808 SurePath specimens were collected from women who were referred to our institution from 01/2000-4/2011 for abnormal Pap tests. The patients' average age was 36.5 years (range 19-85 yr). The cytologic interpretations included NILM (n=497), ASCUS (n=48), ASC-H (n=9), AGC (n=2), LSIL (n=165), LSIL-H (n=27), HSIL (n=56), adenocarcinoma (n=1) and unsatisfactory (n=3). HPV DNA was extracted from residual SurePath specimens and amplified with polymerase chain reaction (PCR) in the L1 region. HPV genotypes were determined by DNA microarray against 40 HPV subtypes followed by a confirmatory sequencing assay.

Results: Patients with LSIL-H had much higher frequency of high risk HPV (HR-HPV) infection (92%) than those with NILM (52%), ASCUS (72.9%), ASC-H (77.8%), LSIL (74.3%), or LSIL/ASC-H combined (74.4%). The frequency of HR-HPV infection in LSIL-H was strikingly close to that in HSIL (91.1%). HPV 16, the most common carcinogenic HPV type, was present in a much larger fraction of LSIL-H (36%) than in LSIL/ASC-H combined (13.8%), but in a smaller fraction than in HSIL (44.6%). Furthermore, LSIL-H and HSIL had similar fractions of low and intermediate risk HPV subtypes which were lower than in LSIL or LSIL/ASC-H combined. The HPV distribution patterns did not differ significantly between younger (<30 yr) and older (>=30 yr) age groups.

Conclusions: The patients classified as LSIL-H had a higher risk for HR-HPV infection which was similar to patients with HSIL and much higher than those with ASCUS, ASC-H, LSIL or LSIL/ASC-H combined. The differences were independent of patients' age. Recognizing LSIL-H as an independent diagnostic category may help in early identification of a higher risk subgroup in LSIL who may require a management algorithm comparable to HSIL.

461 Frequency and Distribution of 40 HPV Genotypes in Uninsured Latino Women with Abnormal Pap Tests

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Background: Knowledge about the prevalence and distribution of HPV genotypes in cervical premalignant and malignant lesions is crucial to guide development of clinical management strategies and of prophylactic vaccines. The aim of this study was to determine the frequency and distribution of HPV genotypes in an underserved cohort of women.

Design: From 1/2010 to 4/2011, 808 SurePath cervical specimens were collected from uninsured Latino women who were referred to our institution for abnormal Pap tests. The patients' average age was 36.5 years (range 19-85 years). The specimens were tested for 40 HPV genotypes by DNA microarray and sequencing assay.

Results: In this underserved cohort of women, the HPV infection rate was high with frequent multi-strain infection (38.4%). The combined frequency of HPV16/18 was 55.1% in HSIL. HPV6 and 11 were infrequent (2.9% and 1.1%). The frequency of HPV 90 was unexpectedly high (2.4%) and associated with dysplasia (9.4%).

Conclusions: The frequency and distribution pattern of HPV genotypes in this cohort differs from previously published US data. HPV 90, an uncommon genotype in the US was identified in the cohort. Understanding the differences and possible changes in HPV distribution pattern may help guide the development of appropriate preventative and therapeutic strategies targeting underserved population.

Table 1. Frequencies and Distribution of 40 HPV Genotypes in Major Cytology Categories

Cytology Diagnosis	No HPV (%)	LR-HPV% (S/M)*	IR-HPV% (S/M)	HR-HPV% (S/M)	Total HPV infection %	Most frequent HR-HPV types (in decreasing order)
NILM (n=497)	9.5	28 (37/23)	13 (15/12)	58 (48/64)	90.5	16, 18, 53, 52, 39, 45, 66, 67, 90, 56
ASCUS (n=48)	8.3	28 (32/27)	8 (0/9)	64 (68/62)	91.7	16, 18, 45, 51, 52, 53, 42, 35, 59, 67
ASC-H (n=9)	11	0 (0/0)	33 (20/43)	67 (80/57)	89	31, 53, 18, 39, 52, 58, 66
LSIL (n=167)	1.2	24 (23/24)	18 (17/18)	59 (60/58)	98.8	53, 56, 16, 18, 66, 58, 39, 51, 67, 82
LSIL-H (n=25)	0	15 (0/24)	6 (11/3)	78 (89/72)	100	16, 58, 51, 45, 39, 31, 53, 67, 18, 33
HSIL (n=56)	1.8	18 (10/22)	5 (0/7)	77 (90/69)	98.2	16, 31, 18, 45, 39,56, 58, 59, 53, 43
Total‡	7.1	26 (31/24)	13 (13/13)	62 (56/63)	92.9	16, 18, 53, 56, 39, 58, 45, 52, 66, 67

*Percentage of HPV infection (Single strain infection/Multi-stain infection); LR-HPV, IR-HPV and HR-HPV represent low, intermediate and high risk HPV, respectively. ‡ include rare AGC (2) and cancer (1) cases (not shown).

Dermatopathology

462 microRNAs as Prognostic Biomarkers in Malignant Melanoma

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Background: MicroRNAs (miRs) are important regulatory molecules. Many recent advances have shown their dysregulation in cancer. Our laboratory has previously reported altered expression of key miRs in melanoma. To develop a further understanding of the clinical importance of such miRs, we have assembled a cohort of primary melanoma patients.

Design: The Agilent miRNA microarray platform was used to generate global miRNA expression profiles for 66 primary melanoma tissue samples. The tumours were from single institution cases, with uniform treatment and follow-up protocols for which clinical data were available. Using supervised analyses, we looked for association of expression level for each miR with pathological (Breslow depth less than or equal to 2mm versus greater than 2mm and low mitotic count versus high mitotic count) and clinical (no metastatic progression versus presence of distant or regional metastasis and alive versus deceased) endpoints.

Results: We identified numerous miRs whose expression level appeared associated with both clinical and pathological endpoints, in most cases showing lowered expression in the more aggressive disease. Of particular interest, we found that expression of miR-150 as well as members of the miR-200 family were significantly associated with the pathological endpoints measured. These miRs were relatively downregulated in thicker melanomas and those with high mitotic rates compared to the thinner or less mitotically active tumours. When clinical endpoints were assessed, lower expression of miR-150 was also strikingly correlated with death and with the presence of metastatic disease.

Conclusions: We conclude that miR expression levels measured in the primary diagnostic lesion can be used to prognosticate clinical outcome in melanoma. The miR-200 family and miR-150 appear to be most promising in this regard. We are currently investigating a role for these miRs and their putative target mRNAs in melanoma progression and outcome, using experimental, statistical and bioinformatics approaches.

463 High ALDH1 Expression Correlates with Better Patient Outcomes in Tumorigenic Malignant Melanoma

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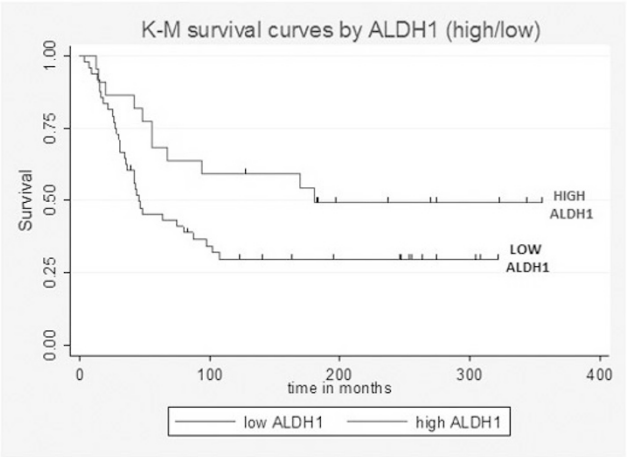
Background: Aldehyde dehydrogenase 1 (ALDH1) is a proposed biomarker of stem cells in certain human cancers. ALDH1 expression has been correlated with poor patient outcomes in breast cancer, but better patient outcomes in ovarian cancer, and its prognostic importance in malignant melanoma is unknown. ALDH1 expression in melanoma is studied using a tissue microarray and immunohistochemistry in a series of 68 patients with long-term follow-up.

Design: 68 tumorigenic melanoma patients from the Pigmented Lesion clinic at the University of Pennsylvania, with comprehensive clinical and pathologic data and at least 10 years of follow-up, were used to construct a tissue microarray. Three to four cores were procured from each lesion. Immunohistochemical staining for ALDH1 was performed and its expression analyzed by two independent readers (RMA, XX) using a modified histological score (H-score) based on staining percentage and intensity, with a maximum score of 300. Survival time was defined as the time between diagnosis and melanoma-specific death (MSD). Kaplan-Meier survival curves were computed for the MSD times. An H-score threshold of 80 maximizes the log-rank test statistic between two resulting ALDH1-groups.

Results: For the cohort, the median age at diagnosis was approximately 59 years and the median melanoma thickness 3.25 mm. The overall percentage of MSD within 10 years was 62%. Using univariate logistic regression, a low (<80 H-score) ALDH1 score showed 3.7-fold increase in risk for MSD within 10 years when compared to high (>80) ALDH1 levels (p= 0.017). Kaplan-Meier survival curves for the two groups based on ALDH1 expression revealed 72% of those with low ALDH expression had MSD compared to only 40% in the group with high ALDH1 expression. Median survival time was 44.1 months and 180.9 months for patients with low and high ALDH1 expression, respectively.



Conclusions: These findings suggest that ALDH1 expression in malignant melanoma has a favorable effect on patient survival, similar to findings seen in ovarian carcinoma and in contrast to findings seen in breast cancer. Further study is needed elucidate the true function of this enzymatic protein in melanoma progression.

464 Use of Anti-PhosphohistoneH3 Immunohistochemistry To Determine Mitotic Rate in Melanoma and Its Correlation with the Lymph Node Status

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Background: Mitotic figure (MF) counting is one of the objective criteria for staging of malignant melanomas according to the AJCC staging. Sometimes rarity of MFs in thin melanomas can make the determination of the mitotic index a very time-consuming task. Moreover there is high interobserver variability in differentiating MF from apoptotic cells.

Design: We tested the utility of the mitosis-specific marker phosphohistone-H3 (PHH3) to enhance rapid recognition of MFs and quick reliable staging of melanomas. One hundred and forty archival melanomas representing variable subtypes and Breslow depths were reclassified according to current AJCC criteria based on mitotic counts labelled by PHH3. The slides stained by hematoxylin and eosin and immuno stains were evaluated by two pathologists and two residents.

Results: Anti-PHH3-labeled MFs were easily seen and permitted quick identification of the area(s) of highest mitotic activity. Because of the higher sensitivity of the immunohistochemical stainings for MFs, average mitotic counts per mm^2 were higher with PHH3 in contrast with H&E in our study. The precise distinction of MFs from apoptoses and the visualization of the distribution of MFs uncovering mitotic hotspots, even at low magnification, turned out to be major advantages of this mitotic marker and less consumption of pathologist's time to count mitotic figures. To this date we are reporting the largest cohort of melanoma studies with PPH3 staining and correlation of outcome affecting management in terms of lymph node status. We also provide 2-3 years clinical follow up of these patients who developed lymph nodes metastases after the primary diagnosis.

Conclusions: Anti-pHH3 immunostain may prove to be superior to the traditional H&E technique for quantifying mitotic figures in future. However careful interpretation of the stain and awareness of the different stages of mitoses along with pitfalls may be necessary for the pathologist to interpret this immuno stain in the right context.

465 Mammary and Extramammary Paget Disease: Clinicopathologic Correlation and Immunohistochemical Analysis

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Background: While mammary Paget disease (MPD) is usually associated with an underlying breast carcinoma, the majority of extramammary Paget disease (EMPD) do not have an underlying malignancy. However, both diseases share similar tumor morphology and the same intraepidermal tumor growth pattern. We examined the clinicopathologic features of MPD and EMPD and compared the expression of androgen receptor (AR), estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (Her2), gross cystic disease fluid protein (GCDFP-15), E-cadherin, and Ki-67 between the two neoplasms.

Design: A retrospective review of all biopsy-proven cases of MPD and EMPD diagnosed at our institution between 2002 and 2010 was conducted. Immunohistochemical studies for AR, ER, PR, Her2, GCDFP-15, E-cadherin, Ki-67 were performed on tissue from the paraffin block. The clinical characteristics, histologic features, and biomarkers were compared between cases of MPD and EMPD.

Results: Nine MPD cases and 6 EMPD cases were identified. The extramammary tumor sites included penis, scrotum, groin, vulva and perianal area. The clinicopathologic data and immunohistochemical profile of these cases are shown in the following table.

	Mammary Paget Disease (N=9)	**Extramammary Paget Disease (N=6)**
Mean Age (years)	61 (range 40-78)	69 (range 56-82)
Sex	All female	Male: 5; female: 1
Underlying carcinoma	absent: 2	absent: 5
	in-situ carcinoma: 2	present: 1
	invasive carcinoma: 5	
AR	Positive: 5; negative: 4	Positive: 4; negative: 2
ER+/PR+/Her2-	1	0
ER+/PR+/Her2+	0	0
ER-/PR-/Her2+	6	1
ER-/PR-/Her2-	1	5
GCDFP-15	Positive: 2; negative: 7	Positive: 2; negative: 4
E-cadherin	All positive	All positive
Ki-67	>10%: 3; ≤10%: 6	>10%: 3; ≤10%: 3

Conclusions: MPD and EMPD (with and without underlying carcinoma) shared similar biomarker expressions for AR/ER/PR and comparable immunohistochemical profiles for GCDFP-15, E-cadherin and Ki-67. However, the majority of MPD cases demonstrated overexpression of Her2, while the majority of EMPD cases were triple negative. This finding may have therapeutic implications.

466 Vulvar Adnexal Neoplasms: A Thirty-Two Year Single-Institution Review

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Background: The incidence of various adnexal neoplasms occurring in the vulvar region has not been reported.

Design: A retrospective review of the case files at our institution was performed (1978-2010).

Results: A total of 194 vulvar adnexal neoplasms were identified. The majority of these neoplasms were benign (136/194, 70.1%) with hidradenoma papilliferum being the most common followed by syringoma and various types of cysts. Malignant adnexal neoplasms comprised the remaining 29.9% (58/194) with Extramammary Paget Disease being the most common.

Benign Vulvar Adnexal Neoplasms (n=136; Total n = 194)

Type	Number of Cases (% of Benign, % of Total)	Age Range, years (Median, years)
Hidradenoma Papilliferum	80 (58.8%, 41.2%)	24-80 (50)
Syringoma	29 (21.3%, 14.9%)	12-74 (51)
Cysts*	13 (9.5%, 6.7%)	20-73 (51)
Poroma	4 (2.9%, 2.1%)	27-77 (56)
Dilated Pore of Winer	2 (1.5%, 1%)	25-56 (40.5)
Spiradenoma	2 (1.5%, 1%)	60-71 (65.5)
Apocrine Tubular Adenoma	1 (0.7%, 0.5%)	60
Cylindroma	1 (0.7%, 0.5%)	71
Hidradenoma	1 (0.7%, 0.5%)	73
Sebaceoma	1 (0.7%, 0.5%)	46
Sebaceous Trichofolliculoma	1 (0.7%, 0.5%)	26
Trichoepithelioma	1 (0.7%, 0.5%)	64

* Cyst subtypes: Epidermoid cyst (n=6), Pilar cyst (n=2), Pilomatricoma (n=2), Trichilemmal cyst (n=2), Hidrocystoma (n=1).

Malignant Vulvar Adnexal Neoplasms (n=58; Total n = 194).

Type	Number of Cases (% of Malignant, % of Total)	Age Range, years (Median, years)
Extramammary Paget Disease (EPD)*	49 (84.5%, 25%)	53-92 (75)
Basal Cell Carcinoma	2 (3.4%, 1%)	80-82 (81)
Sebaceous Carcinoma	2 (3.4%, 1%)	71
Adenocarcinoma, Mucinous	1 (1.7%, 0.5%)	71
Adenocarcinoma, NOS	1 (1.7%, 0.5%)	46
Adenoid Cystic Carcinoma	1 (1.7%, 0.5%)	36
Eccrine Carcinoma	1 (1.7%, 0.5%)	70
Spiradenocarcinoma	1 (1.7%, 0.5%)	50

*EPD, subset with invasive component: 14/49, 24% of malignant neoplasms, 7.2% of total.

Conclusions: This review provides insight into the diversity of adnexal neoplasms occurring in the vulva and their relative frequency. Although several entities predominate, the spectrum of neoplasms observed in this review reflects that which is

seen systemically and correlates with the relative abundance of apocrine, apoeccrine and anogenital mammary-like glands present in the skin and modified mucosal surfaces of the vulva.

467 Exploration of a Genotype-Phenotype Correlation in a Panel of Metastatic Human Melanoma Cell Lines

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Background: Metastatic malignant melanoma remains an incurable disease, but recent advances in innovative therapeutic modalities such as molecular targeted therapy offer exciting new opportunities. These advances have created the clinical need to assess the genetic makeup of tumors. Although genetic testing is becoming widely available it would be an advantage to rely on more widespread technologies, like morphology and immunohistochemistry (IHC) to screen melanomas for common mutations. The focus of our study was to establish if any IHC staining pattern could be used to infer the genotype of melanoma cell lines.

Design: We tested 11 melanoma and 1 dermal fibroblast cell line that we had previously analyzed with a Qiagen qBiomarker™ Somatic Mutation PCR Array interrogating common point mutations in the AKT1, BRAF, cKIT, KRAS, HRAS, NRAS, MEK1, PIK3CA and PTEN genes (Qiagen). We than stained our cells for melanocytic markers (gp100, MELAN-A, tyrosinase), cancer testis antigens (NY-ESO-1, MAGE-A1), metalloproteinases-1 (MMP-1) and protease activated receptor -1(PAR1), using IHC methodology on agar embedded cell pellets.

Results: Three cell lines were wild type for all 85 mutations tested. Eight cell lines harbored various mutations, with all 8 including the common BRAF V600E. Most of the tested markers showed a highly heterogeneous expression with no correlation between IHC staining pattern and underlying genotype. Protease activated receptor -1(PAR1) was consistently strongly positive in all cell lines. Because many of the mutations present in our cell lines act primarily on the MAPK pathway we also analyzed the levels of pERK1/2, pMEK1/2 and pBRAF as a surrogate marker for the activation status of the pathway. Again no positive correlation could be made between the phosphorylation status of key molecules in the pathway and the IHC phenotype.

Conclusions: In light of these results, changes in expression levels of specific molecules upon inhibition at various points of the MAPK pathway might be more revealing of its importance in their regulation. Future studies will help us determine what phenotypic changes might be expected in patients that are being treated with new drugs targeting this pathway.

468 Diagnostic Utility and Comparative Immunohistochemical Analysis of MITF and SOX10 in Melanoma In-Situ: A Clinicopathological and Immunohistochemical Study of 50 Cases

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Background: The diagnosis of melanoma in-situ may be challenging on purely histological grounds in scant biopsy material. In addition, melanoma in-situ may occasionally be confused with intraepidermal melanocytic hyperplasia in sun damaged skin. Sry-related HMG-BOX gene 10 (SOX10) is a transcription factor involved in regulating the promoter of microphthalamia-associated transcription factor (MITF) which is involved in the development of melanocytes. Both MITF and SOX10 are nuclear immunostains, facilitating diagnosis over cytoplasmic staining in other melanocytic markers such as MART-1. SOX10 has been shown to have superior sensitivity in desmoplastic melanoma when compared to MITF as well as increased specificity, as SOX10 expression was confined to melanocytes while MITF expression was seen in histiocytes and fibroblasts. In addition, SOX10 has been shown to exhibit stronger staining in benign melanocytic proliferations when compared to superficial spreading melanoma and nodular malignant melanoma. The purpose of this study was to compare the immunohistochemical staining characteristics of MITF and SOX10 in cases of melanoma in-situ and actinica keratosis with melanocytic hyperplasia to characterize their immunoprofile and diagnostic utility.

Design: A total of 70 cases were studied, including 50 cases of melanoma in-situ and 20 cases of actinic keratosis. The antibodies employed were MITF and SOX10. Appropriate positive and negative controls were run concurrently for each marker studied.

Results: All cases of melanoma in-situ showed strong nuclear positivity for MITF (50/50). All cases of melanoma in-situ showed positivity for SOX10; however, the proportion of atypical melanocytes showing strong nuclear positivity was variable, and did not approach that seen in MITF. There was no expression of either MITF or SOX10 in adjacent pigmented keratinocytes in the cases of actinic keratosis examined.

Conclusions: In summary, MITF exhibits superior sensitivity over SOX10 in cases of melanoma in-situ. In addition, both MITF and SOX10 can be used to differentiate melanoma in-situ from actinic keratosis with lentiginous hyperplasia. MITF is an effective immunostain for the identification and quantification of melanocytes in the setting of melanoma in-situ, espcially in cases where there is limited tissue and in cases where ther is actinic damage with intraepidermal melanocytic hyperplasia.

469 New Prognostic Markers in Merkel Cell Carcinoma

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Background: Merkel cell carcinoma (MCC) is a highly aggressive neuroendocrine carcinoma of the skin that usually occurs on sun-exposed areas in elderly patients. The incidence of this rare tumor is increasing rapidly. The aim of this study was to identify prognostic markers of metastasis, using a panel of immunohistochemical markers recognizing oncogenic feaures of the neoplastic cells, as compared with classical clinicopathological criteria.

Design: Immunohistochemical analysis was performed using antibodies directed against p53, p63, VEGF, NM23 and Ki67 in a series of 33 cases of consecutively diagnosed MCC. Clinical date, follow-up and histopathologic parameters (size, mitoses, vascular invasion, lymphocytic infiltration, vascular invasion) were also evaluated. The significance of pathologic data and the expression of the different markers was evaluated using the chi-square test.

Results: A significant inverse correlation was found for NM23 expression in comparison with regional lymph node metastasis and /or hematogenous spread. A 75% of the cases negative for NM23 showed metastatic spreading while that 39% of the cases immunoreactive for nm23 showed metastasis. No association could be established between the NM23 expression and the tumor size, mitotic index, lymphocytic infiltration and angioinvasion. A statistically significant correlation between the immunoreactivity for VEGF and the metastasis tumor spread was found, with 8 of the 12 (66,6%) cases positive for VEGF showing lymph node or/and solid organ metastasis and only a 33,3% positive cases didn´t show metastasis. The only histopathologic parameter associated with metastatic potential was the tumor size.

Conclusions: Date from the current study indicate that VEGFR expression and loss of immunoreactivity for NM23 are associated with an increased risk of metastatic spread in MCC.

470 Intense Micropthalmia Transcription Factor (MITF) Expression Is a Marker of Mastocytosis

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Background: Micropthalmia-associated transcription factor (MITF) is a basic helix-loop helix leucine zipper transcription factor that is critical for the development of melanocytes, osteoclasts and mast cells. It is frequently used in dermatopathology as a marker of melanocytic lesions as melanocytes intensely express this marker. Recently it has been demonstrated the activating mutations in codon 816 of the tyrosine kinase receptor KIT, found in the majority of patients with systemic mastocytosis, markedly upregulates MITF expression. We sought to determine if overexpression of MITF by immunohistochemistry could be used to evaluate neoplastic mast cell disorders.

Design: Archival formalin-fixed paraffin embedded (FFPE) tissue blocks from patients with mast cell lesions (cutaneous, bone/soft tissue and bone marrow) at this institution were retrieved for MITF immunohistochemistry (C5/D5 IgG1 clone) using standard methods (Ventana Benchmark XT). Nuclear and cytoplasmic expression of MITF was semi-quantitatively scored (intensity: 0-negative, 1+- minimal, 2+- moderate, 3+- strong; extensiveness in target cells 0- 0%, 1- <50%, 2- 50%-75%, 3- >75%). Pertinent clinicopathologic information was recorded. Negative controls consisted of 52 cases from various anatomic sites stained with MITF to assess for background mast cell staining.

Results: A total of 7 patients with adequate tissue blocks were identified for MITF staining (age range 11months- 81 years, mean 54.6 years, M:F 2:5) from different anatomic sites (cutaneous- urticaria pigmentosa n=2, diffuse cutaneous n=1), bone/soft tissue (mastocytoma of bone n= 1), bone marrow n= 3 (systemic mastocytosis n=2, atypical mastocytosis associated with myelodysplastic syndrome n=1). 5 of the 7 cases showed strong staining in >75% of the mast cells (3+); the two negative cases representing the mastocytoma of bone and urticaria pigmentosa in an 11 mo. old. No significant nuclear or cytoplasmic staining (only minimal 1+ intensity) was observed in any of the 52 negative control cases.

Conclusions: Intense extensive nuclear expression of MITF is present in the majority of mast cell tumors (5/7); such staining may be a reliable method to distinguish benign from neoplastic mast cells. Furthermore, intense MITF by mast cell neoplasms is a potential pitfall in the diagnosis of melanocytic lesions. A larger study correlating MITF expression with mast cell phenotype and activating C-kit mutation status is needed to clarify the significance of intense MITF expression in mast cell neoplasms.

471 Non-Infectious Vulvitis: A Histopathologic Review and Classification of 183 Cases

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Background: The vulva is susceptible to a variety of cutaneous and mucosal inflammatory disorders. While well-developed lesions are easy to recognize, most inflammatory vulvar biopsies are challenging due to histologic overlap among early lesions and under-recognition of rare entities. We aim to identify subtle features that help in classifying these cases.

Design: BIDMC archive was searched for non-neoplastic and non-infectious vulvar biopsies. A total of 188 cases were reviewed by the authors blinded to the original diagnoses. Final diagnoses were based on consensus among the authors' and the original pathologist's impressions, and clinical correlation where available. Associations between histologic features and diagnoses were analyzed by χ^2 test.

Results: Five cases were excluded due to presence of Corynebacteria (4 cases) and Herpesvirus (1 case) upon review. Twenty-two cases (12.0%) show evidence of two concurrent processes. A limited differential diagnosis is rendered in 15 cases (8.2%). Conditions encountered include: Eczema (22.4%), lichen sclerosus (LS) (38.8%), lichen simplex chronicus (LSC) (29.0%), Zoon's vulvitis (7.7%), Behcet's (2.7%), hidradenitis (2.2%), ruptured cyst (1.6%), psoriasis (1.1%), radiation dermatitis (1.1%), sebopsoriasis (1.1%), seborrheic dermatitis (1.1%), ulcer NOS (1.1%), and lichen planus (0.5%) (Total>100% due to multiple diagnoses in some cases). Early LS and Zoon's vulvitis are commonly part of a differential diagnosis (6 cases each). LS is significantly associated with subepithelial wiry fibrosis with lymphocyte entrapment ($p<0.0001$). Eosinophils are seen in 44.7% of LS. LSC is associated with zones of pale epithelium ($p<0.0001$)

and prominent stromal fibroblasts (p=0.0004). Fifty percent of Zoon's vulvitis were originally misdiagnosed. Basal keratinocytic crowding with increased N:C ratio is a common finding (92.9%) in Zoon's vulvitis.

Conclusions: Early LS may be confused with LSC, eczema, and Zoon's vulvitis; a useful feature is subepithelial wiry fibrosis with lymphocyte entrapment. The frequent finding of eosinophils in LS suggests a component of hypersensitivity. Zones of pale epithelium and prominent stromal cells help in diagnosing subtle LSC. Zoon's vulvitis tends to be underdiagnosed; while the number of plasma cells may vary, the finding of basal crowding, in combination with more typical features such as intraepithelial neutrophils and mild spongiosis, aids in its diagnosis. We also propose an algorithm to help in classifying vulvitis.

472 The Diagnostic Utility of Novel Sebocyte Maturation Markers ABHD5, PGRMC-1 and Perilipin in Differentiating Sebaceous Carcinoma from Basal Cell Carcinoma with Clear Cell Features

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Background: Sebaceous carcinoma (SebCA) is an uncommon adnexal tumor that occurs predominantly in the ocular and head/neck regions. Distal metastases affect up to 25% of patients, and the overall 5 year mortality is ~20%. Histologically, sebaceous carcinoma consists of sebocytes at varying stages of differentiation, with more differentiated sebocytes at the center of tumor nests. Depending on the degree of sebaceous differentiation, it can be difficult to distinguish SebCA from basal cell carcinoma (BCC). Ancillary markers that separate SebCA from its mimickers are limited. In this study, we sought to identify novel and reliable immunohistochemical markers of SebCA. Specifically, we sought to test if antibodies against ABHD5, PGRMC-1 and Perilipin, proteins involved in either lipogenesis or regulation of lipid synthesis, can be utilized for this purpose.

Design: Archival surgical pathology specimens were retrieved, including 25 cases with diagnosis of SebCA, 17 BCC with clear cell features (BCC-CC) or sebaceous differentiation. Immunostains were performed on FFPE sections using anti-ABHD5, PGRMC-1 and Perilipin antibodies.

Results: First, we demonstrated the distinct localization pattern of ABHD5, PGRMC-1 and Perilipin within normal sebaceous lobules. Second, we showed that ABHD5, PGRMC-1 and Perilipin can reliably differentiate SebCA from its mimickers, namely, BCC-CC. In SebCA, PGRMC-1 showed patchy/organoid distribution and highlighted early differentiating sebocytes; ABHD5 expression was markedly diminished and reduced to a dot-like pattern; and anti-Perilipin diffusely highlighted the majority of intracytoplasmic lipid storage vacuoles in SebCA. In BCC-CC, PGRMC-1, ABHD5 and Perilipin were not expressed. In tumors with sebaceous differentiation, the antibodies highlighted only the areas of sebaceous differentiation in a pattern similar to normal sebaceous lobules (Table 1).

Table 1

	SebCA	BCC	Total
PGRMC +	20	2*	22
PGRMC -	1	15	16
Total	21	17	p=<.0001
Sensitivity	0.95		
Specificity	0.88		
Perilipin +	20	2*	22
Perilipin -	2	15	17
Total	22	17	p=<.0001
Sensitivity	0.91		
Specificity	0.89		
ABHD5 +	22	2*	24
ABHD5 -	3	15	18
Total	25	17	p=<.0001
Sensitivity	0.88		
Specificity	0.88		

* with bona fide sebaceous differentiation

Conclusions: PGRMC-1, ABHD5 and Perilipin highlight sebocytes at various maturation stages in sebaceous glands. These stains can successfully differentiate SebCA from BCC-CC. In addition, our data suggest that sebaceous carcinoma is a tumor with disordered sebocyte maturation and lipid synthesis.

473 The Utitily of Nestin and Sox2 Immunostains in Distinguishing Desmoplastic Melanoma and Dermatofibrosarcoma Protuberans from Excision Scar

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Background: The evaluation of re-excision specimen for residual desmoplastic melanoma (DM) and dermatofibrosarcoma protuberance (DFSP) is a common practice in pathology but is often complicated by the presence of scar tissue. DM is a rare variant of melanoma (MM) that is often amelanotic and neurotropic, and DFSP is a locally aggressive spindle cell tumor of low to intermediate grade malignancy. DFSP has a tendency to local recurrence but rarely metastasizes, and DM is also notoriously recurrent, especially in the presence of neurotropism in original lesion, likely reflecting incomplete excision. Re-excision is often performed after initial excision to ensure no neoplastic cells remain. However, the evaluation of re-excision specimen for residual DM and DFSP can be diagnostically challenging due to the presence of admixed fibroblasts, histiocytes and cells with nuclear atypia in scar tissue, all of which can morphologically mimic DM and DFSP. It has recently been shown that the embryonic stem cell marker Nestin is strongly expressed in MM and DFSP. Sox2, one of the key transcription factors in maintaining pluripotency of stem cells, binds the enhancer region of Nestin and is also expressed in MM. In this study, we sought to test the diagnostic utility of Nestin and Sox2 in differentiating DM and DFSP from re-excision scar.

Design: A total of 40 specimens were retrieved from our archives, including: 14 MM (7 pure DM; 7 spindle cell melanoma [SCM] with DM features), 8 DFSP and 23 scar (5 from above MM cases). Immunostains were performed on FFPE tissue sections using anti-Nestin and anti-Sox2 antibodies.

Results: Cytoplasmic Nestin and nuclear Sox2 were strongly positive in 12 MM (86%). There was weak Nestin in 1 (7%) and negative Sox2 in 2 cases (14%). Nestin highlighted majority of DFSP (n=7/8), while nuclear Sox2 was absent in DFSP. Of all the scar tissue tested, both Nestin and Sox2 were negative. Even though Nestin also highlighted vascular endothelial cells, the staining pattern of vasculature was readily distinguishable from tumor cells. Of note, in 1 DM re-excision specimen that was originally diagnosed as dermal scar, Nestin and Sox2 strongly highlighted one focus of spindle cells with malignant nuclei. Subsequent evaluation of this focus on H&E section confirmed that this represented residual DM.

Conclusions: We demonstrated that the embryonic stem cell marker Nestin is a highly reliable markers in distinguishing DM and DFSP from excision scar. In addition, Sox2 is strongly expressed by DM and can differentiate it from excision scar as well.

474 Diagnostic Value of Neural Progenitor Cell Markers Nestin and Sox2 in Melan-A Negative and HMB-45 Negative Melanoma

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Background: Malignant melanoma (MM) accounts for less than 5% of skin cancers but ~80% of skin cancer death. Histologically, MM exhibits a wide range of cytologic and architectural features that mimic a variety of tumors, making the diagnosis of MM one of the most challenging tasks in surgical pathology. Established immunomarkers of melanocytic differentiation, including Melan-A and HMB45, have been widely used to differentiate MM from its mimickers. Unfortunately, it is well-documented that melanoma can lose these differentiation markers at various phases of growth. HMB45 is lost in up to 50% of metastatic melanoma (MetM), and progressive loss of Melan-A is also observed from stage I to stage IV MM. Furthermore, in the broader category of spindle cell melanoma (SCM), including the rare S-100+ desmoplastic melanoma (DM), the sensitivity of HMB45 and Melan-A is at best 22%, further limiting the utility of these two markers. It has recently been shown that the neural progenitor cell markers Nestin and Sox2 are expressed in MM. In the present study, we sought to test the utility of Nestin and Sox2 in Melan-A/HMB45 negative melanomas, including MetM, DM and the broader category of SCM.

Design: A total of 33 melanoma specimens were retrieved from our archives: 12 MetM, 7 pure DM, 7 mixed SCM/DM, and 7 SCM. Of the 12 MetM, 10 showed complete loss of Melan-A staining, 8 complete loss of HMB45, and the remaining cases showed only focal Melan-A or HMB45 staining. Six of the MetM (50%) were metastatic melanomas of unknown primary site. Immunohistochemical studies were performed on FFPE tissue sections using anti-Nestin and Sox2 antibodies.

Results: Of the 21 DM and SCM, cytoplasmic Nestin was strongly positive in 20 cases (95%). Nuclear Sox2 was also detected in 17 cases (81%). Of the 12 cases of MelanA-/HMB45- MetM, Nestin was strongly and diffusely positive in 10 cases (83%) and patchy in 2 cases (17%). Sox2 was strongly positive in 9 cases (75%) and negative in 2 cases (17%).

Conclusions: In this study, we have shown that despite the loss of established melanocyte-specific markers in MetM, expression of neural stem cell markers Nestin and Sox2 persists. This immunophenotype suggests that some melanomas may adopt a more progenitor-cell like state with disease progression. Diagnosing MetM that do not express the established differentiation melanocytic markers is particularly challenging to practicing pathologists. Our results provide evidence that Nestin and Sox2 can be powerful adjuncts in diagnosing Melan-A/HMB45 negative, neural progenitor-cell like MetM, as well as DM/SCM.

475 Embryonic Stem Cell Markers Nestin and Sox2 Can Differentiate Metastatic Melanoma from Nodal Melanocytic Nevi and Serve as a Powerful Diagnostic Adjunct in Sentinel Lymph Node Evaluation and Melanoma Staging

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Background: Sentinel lymph node (SLN) evaluation is a critical component of the pathologic staging of malignant melanoma (MM), and lymph node status provides one of the most powerful predictors of recurrence and survival. Using melanocytic markers on SLN enhances the sensitivity of melanoma detection. However, these stains have limited specificity in differentiating melanoma metastases from nodal nevus especially when the focus in question is small. It has been reported that 8% of lymphadenectomies harbor melanocytic nevi, and this percentage is higher in melanoma patients. Of the commonly used melanocytic markers, Melan-A and S-100 are indiscriminately expressed in melanoma and nevus cells, while S-100 highlights dendritic cells, making the interpretation of this marker in lymph nodes especially confusing. It has recently been shown that the embryonic stem cell marker Nestin is strongly expressed in MM and is associated with melanoma progression. Sox2, a key transcription factor in maintaining pluripotency of stem cells, binds the enhancer region of Nestin and is also expressed in MM. In this study, we sought to test the diagnostic utility of Nestin and Sox2 in differentiating metastatic melanoma (MetM) from nodal melanocytic nevi (nMN).

Design: A total of 39 specimens were retrieved from our archives: 21 lymph nodes (LNs) with MetM, 18 LNs with nMN from 18 patients (7 with history of melanoma). Immunostains were performed on FFPE tissue sections using Nestin and Sox2 antibodies.

Results: Of the 21 LNs with MetM, 16 showed diffuse 3+ Nestin, and 5 showed rare cells with 3+ Nestin. Nuclear Sox2 was expressed in 13 MetM and strongly highlighted rare cells in 3 cases. By contrast, none of the nMN expressed Sox2 (n=18), 13 nMN showed no Nestin, and 4 nMN showed rare cells with faint (<1+) Nestin. Of note, 1 case that was originally diagnosed as "subcapsular melanocytic rest" based on Melan-A staining and bland cytology showed rare cells with strong 3+ Nestin staining. Follow-up study revealed melanoma recurrence in the same LN region 2 years later.
Conclusions: We have shown that Nestin is strongly expressed in MetM (n=21; 100%), but not nodal nevi (n=17; 100%)($p<0.0001$). Sox2 is also expressed in MetM (n=16; 76%) but not nodal nevi (n=18; 100%)($p<0.0001$). This study provides evidence that embryonic stem cell markers Nestin and Sox2 can effectively differentiate nodal benign melanocytes from melanoma and serve as a powerful diagnostic adjunct in the staging of melanoma patients.

476 Characterization of Neurothekeoma, Plexiform Fibrohistiocytic Tumor and Other Neural and Fibrohistiocytic Neoplasms Using Neural Stem Cell Markers Nestin and Sox2

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Background: Neurothekeoma (NTK) is a rare dermal neoplasm that was first described in 1980 as a benign cutaneous tumor of neural origin due to its resemblance to nerve sheath myxomas. Subsequent attempts at histologic and immunohistochemical characaterization showed that only a subset of NTKs express S-100 and GFAP, and some NTKs can be morphologically indistinct from plexiform fibrohistiocytic tumors (PFT). Recent microarray-based characterization of NTK showed a gene expression profile more similar to cellular fibrous histiocytomas than neural tumors. To date, NTK remains a cutaneous tumor of uncertain histogenesis. In the present study, we sought to test the utility of neural stem cell markers Nestin and Sox2 in distinguishing NTK from its morphologic mimicker PFT, and in classifying other benign and malignant neural and fibrohistiocytic tumors, including schwannoma (SW), malignant peripheral nerve sheath tumor (MPNST), plexiform neurofibroma (pNF), dermatofibrosarcoma protuberans (DFSP), dermatofibroma (DF) and atypical fibroxanthoma (AFX).
Design: A total of 57 archival surgical pathology specimens were retrieved: 8 NTK, 6 PFT, 7 schwannoma, 5 MPNST, 6 pNF, 8 DFSP, 8 DF and 9 AFX. Immunostains were performed on FFPE tissue sections using anti-Nestin and anti-Sox2 antibodies.
Results: Cytoplasmic Nestin was diffusely/weakly positive in NTK and PFT (1-2+), diffusely/strongly positive in subset of pNF (2+), majority of SW (3+) and MPNST (3+), expressed in a patchy distribution in DFSP (2-3+) and negative in DF and AFX. Interestingly, rare giant cells, tumor cell clusters and atypical mitotic figures in AFX were positive for Nestin, and one case of multifocal PFT showed strong (3+) Nestin expression. Sox2 staining was weakly/focally positive in NTK and PFT, strongly/diffusely positive in SW, strongly/focally positive in MPNST, but negative in pNF, AFX, DFSP and DF.
Conclusions: In summary, our findings show that although some tumors of true neural differentiation (such as SW and MPNST) show strong Nestin and Sox2 staining, NTK, PFT and true fibrohistiocytic tumors such as DF and AFX lack such staining profile. In this regard, NTK can not be distinguished from its morphologic mimicker PFT based on Nestin/Sox2 immunostains alone. Interestingly, strong expression of Nestin in recurrent PFT suggests that the upregulation of stem cell markers in fibrohistiocytic tumors can confer more aggressive clinical behavior. Consistent with this, even though AFX is negative for Nestin in the majority of tumor, rare giant cells and bizarre mitotic figures in AFX show Nestin expression.

477 The National Impact of the Rising Incidence of Scalp Melanoma in Central Texas

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Background: Melanoma is a particularly aggressive form of skin cancer that has a fluctuating prognosis depending on the depth of invasion and site of origination. The head and neck region, specifically the scalp, has been described by several authors as the anatomic location with the highest morbidity and mortality, and several epidemiological causes for the poor prognosis have been proposed. These include the presence of hair obscuring visual inspection of the scalp, more intense and prolonged exposure to the sun, as well as increased vasculature and lymphatic drainage allowing for rapid metastasis. With such a significant proportion of the United States population reaching the age distribution of this malignancy, we sought to specifically evaluate melanomas occurring in the hair-bearing portion of the scalp.
Design: To accomplish our stated objectives, we examined 35 patients with scalp melanoma diagnosed between 1976 and June, 2009. A retrospective chart review was performed after permission was obtained from the Institutional Review Board. We collected data pertaining to the patient demographics and tumor characteristics.
Results: The mean age at diagnosis was 69 years. Scalp melanoma was diagnosed in males more than 6 times as frequently as in females. Superficial spreading melanoma was the most common subtype. Seven patients (20%) expired due to metastatic melanoma with a median overall survival of 3.4 years. When compared to patients who survived or those who died of unrelated causes, those patients who expired due to melanoma progression exhibited different characteristics.

Patient Characteristics

	Age	Breslow Depth	Ulceration	Tumor-Infiltrating Lymphocytes
Died of Melanoma	70.4	2.11 mm	2 of 7 (28.6%)	2 of 6 (33.3%)*
Others	69	1.79 mm	3 of 28 (10.7%)	16 of 27 (59.3%)*

*One patient in each group had no mention of tumor-infiltrating lymphocytes in the pathology report, and no tissue was available for review.

Conclusions: The extraordinary increase in scalp melanoma diagnoses and significantly higher mortality rate is troublesome and deserves multidisciplinary recognition and action for the health of our aging population. The mortality rate from invasive melanoma in our series was 2.3 times the published overall rate for all melanomas as provided by Surveillance Epidemiology and End Results (SEER)(20% versus 8.8%). The rising incidence of this more lethal melanoma suggests more rigorous screening is indicated for scalp melanoma.

478 BRAF Mutations Are More Frequent in Younger Patients and Are Inversely Associated with Degree of Dysplasia in Melanocytic Neoplasms

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Background: Mutations in the BRAF gene, particularly the V600E mutation, have been postulated to be early somatic events occurring in common nevi, but data regarding the association have been limited and contradictory. While specific therapeutic inhibitors of BRAF V600E mutants have been developed and have shown great promise in the treatment of metastatic melanoma, the mutation status has not been correlated with the degree of dysplasia, age, gender, or frequency of the mutation in dysplastic nevi.
Design: We identified 129 dysplastic nevi: 43 with mild dysplasia, 44 with moderate dysplasia, and 42 with severe dysplasia. 11 benign compound nevi and 10 melanoma specimens were also examined. Once retrieved from storage, the lesions underwent laser-capture micro-dissection and subsequent semi-quantitative testing for BRAF V600E gene mutations.
Results: The frequency of BRAF mutations and percent of heterozygosity was inversely proportional to the degree of dysplasia ($p<0.04$) (see table). 5/7 patients with personal or familial history of melanoma and multiple dysplastic nevi had BRAF V600E mutations. Patients with BRAF mutations were younger than wild type (median 43 years versus 54 years, $p<0.0001$). Women with BRAF mutations were younger than their male counterparts in each group.

Lesion Characteristics

	Compound Nevi (n=11)	Mildly Dysplastic Nevi (n=43)	Moderately Dysplastic Nevi (n=44)	Severely Dysplastic Nevi (n=42)	Melanoma (n=10)
Mean Age (years)					
Overall	42.4	47.1	45.1	48.7	73.7
Wild type (WT)	41.25	50.25	52.8	52.8	81.1
Mutated	41.75	47.5	44.6	49.7	69.4
Female, WT	44	49.1	46.3	47.7	82
Female, mutated	44.2	41.9	34	36.5	27
Male, WT	33	51.9	62	59.9	80.5
Male, mutated	36	48	37.8	43.5	71
Frequency of BRAF V600E mutations	64%	54%	46%	31%	30%
% heterozygosity	16%	7%	6%	4.8%	4.3%

Conclusions: This data suggests an increased correlation between BRAF mutation status and presence or absence of dysplasia or indeed development of melanoma. However, the presence of BRAF mutations in a subset of young women with dysplastic nevi or melanoma suggests that these mutations in this group may represent one of the many early "hits" in mutagenesis of melanocytic neoplasms.

479 Implications of the 2009 AJCC Melanoma Staging and Classification System for Thin Melanomas

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Background: The TNM staging system for cutaneous melanomas has been revised in the 7th edition of the AJCC staging and classification system. Mitotic rate for thin melanomas was determined to be a stronger predictor of disease free survival and has replaced Clark level of invasion in defining T1 lesions. In addition, a single isolated tumor cell in a sentinel lymph node was determined to be sufficient for defining stage III disease. We report the impact of the new classification system in defining T1 lesions and the status of sentinel lymph node (SLN) biopsy.
Design: The surgical pathology database was searched for cutaneous melanomas from January 1, 2010 to June 30, 2011. Invasive melanomas were reviewed by dermatopathologists with all reported histologic features included in the analysis. 340 cases were identified of which 139 had Breslow thickness ≤ 1.0 mm. The 139 cases were categorized according to the 2001 and 2009 AJCC classification system. Clark level (CL), Breslow thickness (BT), mitosis (M), ulcer (U), and SLN status was collected. Comparison between groups were made using t-tests.
Results: T1 lesions were redistributed in 41/139 cases according to the 7th edition of AJCC classification system. 18 T1b cases with CL < 4 were identified based on the presence of only mitosis. These cases would have been defined as T1a under the 6th edition. 23 cases with CL ≥ 4 were redistributed to T1a due to absence of mitosis. 46/60 T1b cases had SLN biopsy. 6/46 cases had positive SLN in which metastatic melanoma was detected in 8/11 SLNs examined. 5/8 positive SLNs demonstrated isolated tumor cells (less than 15 melanoma cells) and one SLN with a single positive tumor cell. None of 18 redistributed T1b cases had a positive SLN. Mean BT in lesions categorized as CL 2/3 -M/-U was signigicantly lower ($p<0.0001$)†.

Distribution of Thin Melanomas

	2001 AJCC, 6th Edition		2009 AJCC, 7th Edition ('*' = +SLN)		Mean Breslow Thickness (mm)
	T1a	T1b	T1a	T1b	
CL 2/3 -M/-U	56	0	56	0	0.42†
CL 2/3 +M/-U	18	0	0	18	0.72
CL 2/3 -M/+U	0	1	0	1 (1*)	0.64
CL 2/3 +M/+U	0	4	0	4	0.77
CL 4/5 -M/-U	0	23	23	0	0.60
CL 4/5 +M/-U	0	36	0	36 (4*)	0.74
CL 4/5 -M/+U	0	1	0	1 (*1)	0.60
CL 4/5 +M/+U	0	0	0	0	0
Total	74	65	79	60	

Conclusions: The 7th edition of the AJCC staging system redefined nearly 30% T1 lesions in our sample. The number of T1a cases increased by 5 with a commensurate decrease in T1b cases by 5. The total number of T1b lesions was lower and thus fewer cases met the criteria for SLN biopsy. Cases with positive SLN were defined as T1b according to the 2001 and 2009 classification system.

480 RAS Mutation Analysis of Transformed Mycosis Fungoides Identifies a KRAS G13D Mutation

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Background: Mycosis Fungoides (MF) is the most common cutaneous T-cell lymphoma (CTCL), which, upon progression to tumors and/or large cell transformation (T-MF) may follow an aggressive course, with a mean survival <2 years. The molecular characterization of MF has been generally limited to cytogenetic and gene expression analyses. Mutation analysis of cancer-related genes in MF has rarely been performed. Recently, KRAS G13D and NRAS Q61K mutations in 2 cases each of stage IV CTCL were reported; one was in a lesion of MF. We investigated T-MF for RAS mutations using a Sequenom MassArray Platform.

Design: Ten FFPE tissue samples from patients with tumor (stage IIB: n=7) or nodal (stage IVA2; n=3) T-MF were retrieved from the pathology archive. Clinical and morphologic features were assessed; review of H&E sections confirmed abundant cellularity of samples. Genomic DNA was extracted from 5 micron thick tissue sections. Sequenom Mass Spectrometry Genotyping was used to assess for specific mutations in KRAS codons 12,13,61,117,146, and NRAS codons 12,13,61.

Results: One case showed a KRAS mutation in codon 13 (G13D). The specimen was from a skin tumor in a 28 year old patient with a 12 year history of MF, staged as IIB, who later died of disease. The remaining patients, 7 of whom died of disease, and including 3 with LN involvement (stage IVA2); and 2 with SS (stage IVA1), did not show mutations.

Patient characteristics

Patient #	Age of 1st Dx/sex	Time to death/ LF (years)	Status	Site of bx	stage at bx	RAS mutation
1	57M	1	DOD	skin	IIB	-
2	52M	12	DOD	LN	IVA2	-
3	16M	15	DOD	skin	IIB	+
4	60M	2	DOD	skin	IIB	-
5	23M	15	chronic	LN	IVA2	-
6	49M	14	DOD	skin	IIB*	-
7	74F	11	chronic	LN	IVA2	-
8	60M	2	DOD	skin	IVA1	-
9	68M	9	DOD	skin	IIB	-
10	82M	1	DOD	skin	IIB	-

Dx:diagnosis, LF:last follow up, bx: biopsy, DOD: dead of disease, *subsequently developed sezary syndrome

Conclusions: One of our cases was positive for a RAS G13D oncogene mutation similar to that previously described in a single case of stage IV MF. Features notable about our patient include young age at diagnosis, length of early stage disease, and lack of nodal or blood involvement. While it is difficult to draw definitive conclusions from a limited sample, we hypothesize that RAS mutations in MF may occur as a late event in the context of long-standing disease, and may serve as a marker of disease progression, rather than a cause.

481 Oncofetal Protein IMP3, a Diagnostic Biomarker To Distinguish Microcystic Adnexal Carcinoma from Syringoma

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Background: An accurate diagnosis between microcystic adnexal carcinoma (MAC) and syringoma is essential for patient management. The diagnosis of MAC, especially in superficial biopsies can present a diagnostic challenge. IMP3 is an oncofetal protein associated with aggressive and advanced tumors and is specifically expressed in malignant tumors but not found in benign tissues. The aim of this study was to investigate the expression of IMP3 in MAC and syringoma and determine whether it can serve as a diagnostic biomarker for MAC.

Design: A total of 47cases (excision, n=19; biopsy, n=28), including 12 MACs and 35 syringomas were obtained from the surgical pathology files of a tertiary Medical Center between 2002 and 2011. The diagnoses of all the cases were confirmed by at least two pathologists.

Results: A positive immunohistochemical stain for IMP3 (cytoplasmic staining) was not identified in any of the syringoma cases (0%) but stained positive in 8 of 12 cases (67%) (P<0.001). IMP3 positivity was detected in >50% of tumor cells in 5 (63%) cases and <50% of tumor cells in 3 (37%) cases. The sensitivity of IMP3 for the detection of MAC was 67% and the specificity was 100%.

Conclusions: Our findings indicate that IMP3 is a fairly sensitive and highly specific positive biomarker for MAC. IMP3 is expressed in MAC but not in syringoma, which suggests it may play an important role in the pathogenesis of MAC as it has been shown to play a role in cell proliferation, invasion and migration. The expression of IMP3 in tumor cells can increase the level of confidence in establishing a definitive malignant diagnosis of MAC from syringoma.

482 SSTR2A Is Highly Expressed in Metastatic Uveal Melanoma

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Background: Somatostatin is a neurohormone that is found in both the nervous system and certain peripheral organs. Its biological effects, which include inhibition of cell secretion and regulation of cell proliferation and differentiation, are effected through interaction with the somatostatin receptor (SSTR), of which there are at least five distinct subtypes. SSTRs have been shown to be expressed by several different tumors, including uveal malignant melanoma. Somatostatin receptors represent a potential target for treatment of melanoma, but studies to this date have been inconclusive as to the prognostic significance of SSTR subtype expression. In addition, no prior studies to this date have examined SSTR expression in metastases from uveal melanomas.

Design: Our study consisted of 15 cases of metastases from uveal malignant melanoma. All cases were stained for SSTR2A and SSTR5 (Pierce Antibodies). For SSTR2A, cases were evaluated for positive cytoplasmic, nuclear, and/or membranous staining. Cytoplasmic staining was graded on a three point scale, 0 = no staining, 1 = diffuse weak staining or strong staining in <50%, 2 = diffuse strong staining. Nuclear and membranous staining were evaluated by percentage of cells staining (positive stain, >20%). SSTR5 was evaluated for cytoplasmic staining by percentage of cells staining. The staining pattern was correlated with OctreoScan results.

Results: Cytoplasmic staining for SSTR2A was most common, with 93% of cases staining positive (14/15). 60% (9/60) of cases exhibited nuclear staining for SSTR2A; membranous staining was negligible and was not included in the analysis. 7% (1/15) cases stained positively for SSTR5. Of the cytoplasmic SSTR2A positive cases, 93% were also positive by OctreoScan (13/14). In addition, 45% of these cases were positively correlated in terms of the surgical specimen site and the body site of OctreoScan positivity (5/11; two cases were unable to be determined). The case negative for cytoplasmic SSTR2A was also negative by OctreoScan. 100% (7/7) of cases which positively stained for nuclear SSTR2A were also positive by OctreScan; 57% (4/7) of these had positive site correlation. The SSTR5 positive case was also positive by OctreoScan, with positive site correlation.

Conclusions: Somatostatin receptors are commonly expressed in metastases of uveal melanoma. Staining for SSTR2A is correlated with OctreoScan results for somatostatin receptors. These receptors represent a potential target for treatment of metastatic uveal melanoma.

483 Correlation of Immunohistochemistry for HER2 with Bright Field Dual ISH (DDISH) in Extramammary Paget Disease

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Background: Immunohistochemistry (IHC) for HER2 expression is frequently positive in extramammary Paget disease (EMP). Like HER2 positive breast cancer, treatment of EMP with trastuzumab, a monoclonal antibody directed against HER2, has shown antitumoral activity. In the setting of breast cancer, evidence of gene amplification is often required before initiating HER2-targeted therapy. Unlike in breast carcinoma, little work has been done correlating immunohistochemistry for HER2 and *HER2* gene amplification in EMP. We compared IHC with a bright field in situ hybridization assay for determination of HER2 status in EMP.

Design: Cases coded as EMP were retrieved and immunostained for HER2 (4B5) and also evaluated by dual color dual hapten bright field silver in situ hybridization (DDISH), and correlated with IHC expression (Pathway HER2; 4B5 rabbit monoclonal antibody; Ventana Medical Systems) with cell line expression controls. Demographics were also reviewed.

Results: Nine cases were evaluated. 7/9 were women and 6/9 were from the vulva and 3/9 were perianal. 7/9 showed staining by IHC for HER2. Of these cases, 5/7 were 1+, 1/7 was 2+, 1/7 was 3+. *HER2* gene amplification detected by DDISH with protein over-expression, was seen in three cases (one 3+ IHC, one 2+ IHC, and one 1+ IHC), the latter two demonstrating low level, but definitive *HER2* gene amplification.

Conclusions: IHC for HER2 expression in EMP may be a useful adjunctive diagnostic tool. *HER2* gene amplification as assessed by DDISH was inconsistently associated with overt HER2 protein over-expression by IHC. Low level gene amplification may occur in a greater proportion of EMP patients as compared with breast carcinoma, but a larger cohort of patients will be required to fully evaluate this pattern. In the setting of EMP, DDISH may be more sensitive than IHC for detecting patients eligible for trastuzumab based therapy, which could have potential therapeutic implications for patients with unresectable or advanced EMP. Additional cases of EMP need to be evaluated by simultaneous IHC and DDISH.

484 Distinct Patterns of Ciliation in Divergent Classes of Melanocytic Lesions

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Background: Primary cilia are ubiquitous sensory organelles that coordinate tissue development and adaptation. Defects in ciliogenesis are associated with the induction of pro-oncogenic signaling and primary cilia can enhance or repress the development of Hedgehog-dependent cancers; however, the role and fate of these organelles in malignancies such as melanoma, which are driven primarily by other signaling pathways, is unclear.

Design: Using a combination of melanocyte, primary cilium and basal body-specific antibodies in conjunction with confocal microscopy, we examined a total of 87

melanocytic lesions, including 22 common nevi, 16 primary cutaneous melanoma in situs, 16 primary cutaneous invasive melanomas, 8 primary cutaneous nodal metastases, 7 Spitz nevi, and 18 primary mucosal melanomas for the abundance of primary cilia.
Results: Nearly all melanocytes in common melanocytic nevi are ciliated whereas there is a near-complete loss of these organelles in primary cutaneous melanoma in situ, primary cutaneous invasive melanoma, and metastatic melanoma of cutaneous origin. In contrast, the frequency of ciliated melanocytes is reduced in Spitz nevi and variable in primary mucosal melanoma.
Conclusions: In cutaneous melanomas, which are driven by hyperactivation of the Ras-Raf-MAPK signaling axis in the vast majority of cases, the loss of the primary cilium occurs initially during the development of melanoma in situ and is sustained throughout subsequent disease progression. However, in mucosal melanomas, which feature genetics substantially divergent from those of cutaneous melanoma, ciliation is variable. The patterns of ciliation amongst malignant melanoma from distinct anatomic sites further supports the concept that each melanoma subtype may utilize a distinct repertoire of oncogenic signaling pathways and that the primary cilium may serve either tumor suppressive or pro-oncogenic functions, depending on the subtype of melanoma. Spitz nevi, which harbor unique genetic aberrations not observed in common nevi or cutaneous melanoma, retain cilia but at a reduced frequency when compared to common nevi. The pattern of ciliation observed among Spitz nevi would suggest that these nevi arise via mechanisms different than those of common nevi and cutaneous melanoma. Altogether, our findings parallel the reported genetic heterogeneity observed among these diverse lesions and suggest that the presence of the organelle may be an indication of particular driving mutations.

485 Connective Tissue Nevus: A Rare Lesion Analyzed in a Series of 25 Cases

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Background: Connective tissue nevus (CTN) is an unusual benign cutaneous lesion that remains poorly defined in the literature. The purpose of this study was to characterize the clinicopathologic and immunohistochemical features of CTN.
Design: 25 cases of CTN were retrieved from consult files. Clinical, pathologic and immunohistochemical features were evaluated. Clinical follow-up was obtained from medical records and referring physicians. Descriptive statistical analysis was performed.
Results: Sixteen patients were female (64%) and 9 were male (36%), with age ranging from 1.5 months to 58 years (median 10 years). Most patients presented with a slowly growing, painless plaque-like or nodular skin lesion (median size 0.6. cm, grossly). Eleven cases (44 %) arose on the trunk, 9 (36%) on the head and neck, and 5 (20 %) on limbs. All tumors showed poor circumscription and were situated primarily in the reticular and deep dermis, extending into superficial subcutis in 13 cases (52%). The lesion was associated with papillomatous epidermis in 17 cases (70%) and presence of adipose tissue in the reticular dermis in 14 cases (60.9%). All tumors were composed of a disorderly proliferation of bland intradermal fibroblastic cells with palely eosinophilic cytoplasm and tapering nuclei, with no significant cytologic atypia or pleomorphism, arranged in short-intersecting fascicles and entrapping appendages. No mitoses were identified. Immunostains showed positivity for CD34 in 20 of 23 cases (87%) and weak focal positivity for smooth muscle actin (SMA) in 9 of 19 cases (47%). No case stained for desmin (0 of 11 cases). Clinical follow-up was obtained for 13 patients. No tumor recurred locally, even when surgical excision was incomplete. No lesion metastasized.
Conclusions: CTN occurs most commonly as a plaque on the trunk and head/neck of children, involves deep dermis and superficial subcutis, and stains mainly for CD34. CTN most likely represents a localized development anomaly; it is entirely benign and should not be confused with dermatofibrosarcoma protuberans or other neoplasms.

486 Metastasizing "Benign" Fibrous Histiocytoma: A Clinicopathologic Study of 15 Cases

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Background: Cutaneous fibrous histiocytoma (FH) is considered a benign tumor; however, certain types of FH have been shown to have a tendency for local recurrence and there are a few reported cases of metastasis.
Design: 15 cases of morphologically "benign" FH with locoregional or distant metastasis were identified in consult files. Pathologic features and clinical outcome of primary, recurrent and metastatic tumors were evaluated.
Results: 9 patients were male and 6 female; mean age was 42 years (range 3-68). 6 tumors arose on the trunk, 5 on the lower limbs, 2 on the neck and 1 on the upper limb. Primary site in 1 case was unknown. 14 primary tumors involved the dermis; 6 extended into subcutis. Tumor size ranged from 1 to 5cm (mean 3.2). Histologically, primary tumors showed characteristic features of FH, consisting of a polymorphous population of bland spindle and histiocytoid cells with a mixed storiform and fascicular growth pattern and admixed foam cells, multinucleate cells and inflammatory cells. There were 9 cellular, 2 aneurysmal, 1 mixed atypical/cellular, 1 atypical and 1 epithelioid lesions. All tumors showed entrapment of hyalinized collagen bundles. Epidermal hyperplasia was present in most cases. Mitoses ranged from <1 to 13 per 10 HPF. Focal necrosis was seen in 3 primary tumors. Vascular invasion was not seen. Focal or multifocal SMA expression was seen in 5/7 cases and weak focal CD34 expression in 1/8. Tumor cells were negative for pan-keratin and S100. 9 tumors recurred locally, 5 as multiple "satellite" nodules. Time to first recurrence ranged from 6 weeks to 3 years. 1 patient had 3 local recurrences and 1 had 7. The local recurrences of 2 tumors showed increased cytologic atypia. Metastases occurred 0-96 months after diagnosis (mean 27.5) and involved the lungs (11 patients), soft tissues (6), lymph nodes (5) and liver (1). Metastases were morphologically similar to the primary tumors in all cases. 6 patients so far died of disease, with average time to death of 102 months (range 10-216). 2 patients are alive with stable disease 68 and 132 months after initial presentation and 65 and 66 months after lung metastases. 1 patient remains disease-free 7 years after resection of nodal and lung metastases. 1 patient died of unrelated disease 1 year after initial diagnosis.
Conclusions: Metastasis of morphologically benign cutaneous FH, although probably extremely rare, is a clinically aggressive event. Primary tumors tend to be large and cellular but aggressive behavior cannot be predicted on morphologic grounds. Early or frequent local recurrence may warrant close follow up.

487 Genomic Characterization of Merkel Cell Polyomavirus Integration Sites in Merkel Cell Carcinoma

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Background: Merkel Cell Carcinoma (MCC) is a rare cutaneous tumor that has a strong association with Merkel Cell Polyoma Virus (MCPyV). Given its relative rarity, little high-quality frozen tissue is available for traditional genomic characterization. We used targeted next generation sequencing (NGS) to identify viral sequence alterations and human genomic integration sites from formalin-fixed cases of MCC.
Design: We identified 25 cases of previously characterized MCC cases, including 7 cases that tested negative for MCPyV by PCR. 500ng of genomic DNA was extracted from each case and indexed NGS libraries created. DNA was then captured using custom synthesized, biotinylated oligonucleotides that spanned the 5.3Kb MCPyV genome. The enriched DNA was sequenced on both an Illumina GAII and Illumina MiSeq as two indexed pools of 13 samples using 100bp and 150bp paired-end reads, respectively. Data were analyzed using freely-available software.
Results: The 18 MCPyV positive cases yielded an average coverage of 3084x with the GAII and 676x with the MiSeq. Sufficient coverage was obtained in 11 cases for further analysis. In 7 of these cases (63%), MCPyV genomic deletions >100bp were detected. In 8 cases (72%), human integration sites were identified, including 4 within introns (*UBAC2*, *SEMA3D*, *XRCC2*,*FAM53A*) and 4 within non-coding regions. In 5 of 7 (71%) MCPyV-negative cases we detected small fragments of MCPyV, all of which included the same 100bp segment of the large T antigen. Alignments of this area across all organisms demonstrated that region was unique to MCPyV.
Conclusions: The finding that none of the viral integration sites occurred in coding or known regulatory regions argues against a role for insertional mutagenesis of MCPyV in the pathogenesis of MCC. The identification of a small 100bp region within the MCPyV large T region in the majority of PCR-negative cases suggests that this minimal viral region is involved in MCC pathogenesis; it also indicates that a higher percentage of cases of MCC harbor MCPyV than currently recognized and provides a target for more sensitive PCR-based tests to detect the virus. In a broader context, our results indicate that NGS can detect unique, non-recurring deletion and insertion events, demonstrating the utility of the methodology for clinical applications focused on these types of genetic aberrations.

488 Basal Cell Carcinomas of the Vulva Are Unrelated to High-Risk Human Papillomavirus

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Background: Basal cell carcinoma (BCC) of the vulva is rare and may be confused with the much more commonly encountered high-risk human papillomavirus (HPV)-related basaloid squamous cell carcinoma (SCC) of the vulva. Basaloid SCCs are characterized by a diffuse p16 expression pattern which serves as a surrogate marker for high-risk HPV in these tumors. The HPV status of BCCs is not well-established: p16 expression studies are limited and analysis by HPV in situ hybridization has not been previously reported. This study assesses the utility of p16 expression patterns and high-risk HPV detection in a series of vulvar BCCs for distinction of these tumors.
Design: Twelve cases of vulvar BCC identified in the surgical pathology archives from 1996 to 2010 were analyzed. P16 expression was assessed by immunohistochemical analysis and HPV status was assessed by in situ hybridization with a wide-spectrum probe for high-risk HPV types. P16 staining patterns were scored as either diffusely positive (>90% of tumor cells positive) or as patchy positive/negative when a lesser extent of staining or absence of staining was encountered.
Results: The average age of the patients was 80.3 years (range 54-95). All tumors (12/12) demonstrated patchy p16 positivity, with <50% of tumor cells expressing p16 in all cases. None demonstrated the diffuse p16 expression characteristic of high-risk HPV-associated lesions. No high-risk HPV was detected by in situ hybridization (0/12).
Conclusions: The lack of diffuse p16 expression and failure to detect high-risk HPV by in situ hybridization in vulvar BCCs indicate that these tumors are unrelated to high-risk HPV. These ancillary techniques, particularly p16 immunohistochemical analysis which is readily performed in most laboratories, are useful for distinguishing vulvar BCCs from basaloid forms of high-risk HPV-related vulvar SCC. Distinction of these tumors is important due to differences in extent of surgical treatment for these entities.

489 Use of Immunohistochemistry (HMB-45,p16 and Ki-67) in the Diagnosis of Spitzoid Lesions

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Background: Spitzoid neoplasms, which include spitz nevus (SN), atypical spitz nevus (ASN) and spitzoid melanoma (SM), are diagnostically challenging lesions. The histological similarities between SN and other spitzoid lesions may result in considerable problems in differential diagnosis. The aim of this study was to compare immunohistochemical expression of a group of markers including gp 100 (HMB-45), P16 and Ki-67 in spitzoid neoplasms.

Design: Thirty cases of spitz nevi, 19 cases of atypical spitz nevi and 20 cases of spitzoid melanoma were retrospectively selected from the archives of the Department of Pathology at the University of Texas, MD Anderson Cancer Center from the period 1992-2006. The diagnoses were confirmed by two authors including also review of clinical charts for possible events. Immunohistochemistry was performed in each case for gp100 (with HMB45), P16 and Ki-67. HMB-45 was evaluated in melanocytes of 3 different localizations of the lesions (i.e. junctional component, and superficial and deep dermis). Cytoplasmic P 16 intensity and percentage were separately evaluated in the dermis. Proliferative index was calculated by counting the cells that expressed Ki-67 per/mm².
Results: Ki-67 proliferative index was significantly higher in SM compared to SN and ASN ($p<0.0001$). Intensity of cytoplasmic P16 staining was stronger in SN and ASN compared to SM; however the difference between groups were not significantly different. p16 expression percentages was significantly higher in SN and ASN compared to SM ($p<0.001$). The percentage of intraepidermal cells labeled with HMB-45 was not different among groups. However, more cells in superficial and deep dermis were labeled with HMB-45 in SM compared to ASN and SN ($p<0.0001$).
Conclusions: According to our data, spitzoid melanoma shows higher expression of gp100 (with HMB45) and Ki67, and lower of p16 than Spitz nevi and atypical spitzoid lesions. Therefore, immunohistochemical analysis of these markers may help in the diagnosis of spitzoid lesions.

490 Cutaneous Clear Cell Sarcoma: A Study of 3 Additional Cases with Molecular Confirmation

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Background: Clear cell sarcoma (CCS) is a distinctive soft part tumor showing some morphologic and immunophenotypic overlapping with melanoma, hence its time-honored designation of "melanoma of soft parts". Yet, CCS possesses distinctive microscopic features allowing its recognition, including an organoid, spindle cell pattern along to scattered larger "wreath-like" cells. In addition it harbors a unique *EWSR1-ATF1* gene fusion secondary to the t(12;22)(q13;q12) translocation. Recently, CCS have been reported to occur in the skin where it may simulate a broad spectrum of entities, including spindle cell melanoma. In this study we describe 3 new cases of CCS of the skin with confirmatory molecular study.
Design: Three cases of cutaneous CCS were retrieved. The patients were a 12 year-old boy, a 29 year-old woman and a 60 year-old man complaining of nodules occurring in the foot, plant and thigh skin respectively, originally deemed suspicious of spindle cell melanoma. A panel of antibodies against cytokeratins, S100 protein, gp100, and melan A was applied to all cases. In addition all the cases had been tested for the *EWSR1-ATF1* gene fusion/t(12;22)(q13;q12) translocation.
Results: Microscopically all the lesions were nodular proliferations centered in dermis featuring an organoid, spindle cell pattern along to scattered larger "wreath-like" cells. Fibrous septa were recognized. One case was associated with junctional focal melanocytic proliferation. Loose spindle cell nodules with a neuroid appearance were occasionally noticed. A low to moderate mitotic activity was observed as well. Tumor cells were positive for S100 protein (3/3) and gp100 (1/3). FISH analysis demonstrated chromosomal translocation t(12;22) to be present in >80% of tumor cell nuclei. Sentinel lymph node biopsy was negative in 2 patients. Follow up was uneventful in 2 patients; 1 patient developed a lymph node metastasis 5 months after primary tumor excision and was lost to follow up after one year.
Conclusions: Data from our study confirm evidence that malignant dermal tumors similar but not conforming to spindle cell melanoma should raise the suspicion of CCS and prompt investigation of confirmatory gene fusion t(12;22). Although actual patient management does not differ from that of melanoma, including conservative cutaneous excision and possibly a sentinel lymph node biopsy, the distinction between these conditions is especially warranted by the perspective of tailored patient management.

491 *c-myc* in Kaposi's Sarcoma – Incidental or Causative?

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Background: The *c-myc* proto-oncogene, which plays a central role in the regulation of cellular transcription, differentiation and apoptosis, can also promote angiogenesis. This gives *c-myc* a dual, direct and indirect, oncogenic function as tumor growth requires both cell proliferation and uncontrolled angiogenesis. The correlation between the expression of the c-Myc protein and presence of Kaposi's sarcoma-associated viral DNA in Kaposi's sarcoma, an angioproliferative disorder, has not been previously examined.
Design: Formalin-fixed paraffin-embedded archival tissue samples of Kaposi's sarcoma (n=24) were obtained. Immunohistochemical staining for the c-Myc protein was performed using the monoclonal anti-c-myc antibody 9E10.The control group included tissue samples of hemangiomas (n=11) and non-radiation induced angiosarcomas (n=6). Cases were marked as positive if they exhibited either nuclear or cytoplasmic staining. DNA extraction and PCR amplification were performed on all of the cases of Kaposi's sarcoma for presence of Kaposi's sarcoma-associated herpesvirus DNA. Statistical analyses were conducted to ascertain if there was a correlation between the results of immunohistochemical and PCR-based virus DNA detection.
Results: Immunohistochemical analyses revealed positive staining in 13/24 Kaposi's sarcoma cases (54%; nuclear=4; cytoplasmic=4; both=5) with negative staining in the control group. DNA PCR analyses revealed that 20/24 Kaposi's sarcoma cases (83%) were positive for Kaposi's sarcoma-associated herpesvirus DNA. Ten of 24 Kaposi's sarcoma cases (42%) were positive for both, one case (4%) was negative for both. No statistical correlation between c-Myc levels and the presence of viral DNA was observed.
Conclusions: Our findings indicate that the presence of the c-Myc protein, as determined by immunohistochemistry, does not correlate with the existence of Kaposi's sarcoma-associated herpesvirus DNA. Amplification of *c-myc* using more sensitive techniques, such as *in situ* hybridization, is required to ascertain whether it plays a definitive role in the etiopathogenesis of Kaposi's sarcoma.

492 CD30 Positive Lymphomatoid Drug Reactions

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Background: Atypical cutaneous lymphocytic infiltrates of the skin can be induced by drug therapy, defining the concept of the lymphomatoid drug reaction. The most common expression of the lymphomatoid drug reaction is one which resembles mycosis fungoides(MF) including the interstitial granulomatous variant of MF. The latter has fallen under the appellation of drug associated reversible granulomatous T cell dyscrasia. The so called lymphomatoid vascular reaction has been described as a unique morphologic variant of lymphomatoid drug reaction. In this study we present a group of patients with a CD30+ lymphomatoid angiocentric drug reaction, a reaction pattern which closely resembles type A lymphomatoid papulosis.
Design: All cases were encountered in the routine dermatopathology practice of the authors. A total of 9 cases were encountered in which a diagnosis of CD30+ lymphomatoid drug reaction was confirmed based on the temporal association between lesional onset with initiation of drug therapy and subsequent resolution with drug cessation.
Results: The patients presented with a pruritic papular eruption. There were 5 women and 4 men ranging in age from 49 to 76 years of age. In each case, the biopsies demonstrated a similar morphology, namely a superficial angiocentric lymphocytc infiltrate containing many immunoblasts exhibiting variable atypia. Tissue eosinophilia and supervening eczematous changes in the overlying epidermis were not uncommon. In all cases, the angiocentric infiltrate was highlighted by CD3, CD30, and CD4. Cytotoxic protein granule expression or monoclonality were not observed. In all cases there was improvement or complete regression of the eruption upon drug modulation. The drugs were among those most commonly implicated in the lymphomatoid drug reactions, comprising statins (4 cases), ACE inhibitors especially linisopril (5 cases), calcium channel blockers (2 cases), chemotherapy agents (3 cases), HCV associated antiviral therapy (1 case) and antidepressants (1 case).
Conclusions: The CD30 positive lymphomatoid drug reaction poses a diagnostic challenge because of its close resemblance to lymphomatoid papulosis. Differences morphologically include the superficial confinement of the reaction, lack of cytotoxic protein expression amid the atypical immuoblastic cells, and supervening eczematous alterations in the epidermis. A role for combined iatrogenic and endogenous immune dysregulation including polypharmacy is likely important pathogenetically.

493 Clinicopathologic Spectrum of Cutaneous Marginal Zone Lymphoma: Differences between Primary and Secondary Involvement

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Background: Cutaneous marginal zone lymphoma (CMZL) is considered part of the broad group of extranodal marginal zone B-cell lymphomas in the 2008 WHO classification. Distinguishing primary CMZL (PCMZL) from secondary CMZL (SCMZL) is of utmost importance for prognostic and therapeutic approach.
Design: We analyzed the clinicopathologic, phenotypic and genotypic features of a series of 21 patients that showed histopathologic features of CMZL in at least 1 biopsy either at initial presentation or at relapse.
Results: Nine patients were male and 12 were female. Median age was 48 years (range: 23 to 72). Six patients had synchronous or metachronous documented extracutaneous disease. Three cases had a breast MALT lymphoma and other extranodal sites included parotid gland, nasopharynx and bone marrow in one case each, respectively. Morphological and clinical dermatologic features of SCMZL were indistinguishable from cases with PCMZL. All cases showed monotypic plasma cells with immunostains or in situ hybridization for κ and λ; 14 cases showed monotypic κ expression, and 7 cases showed monotypic λ expression. No differences were found between these two groups. Nevertheless, all tumors with primary cutaneous involvement had class-switched heavy chain, with numerous admixed T cells ($p=0.036$) and clusters or small aggregates of dendritic plasmocitoid cells CD123+ ($p=0.013$), whereas IgM positive plasma cells were observed in 5 out of 6 SCMZL, mixed with isolated CD123+ cells and scattered T cells. Moreover T cells in PCMZL, more often express a CD4+ phenotype.
Conclusions: Our observations indicate that PCMZL are distinct from secondary cutaneous forms of extracutaneous MALT lymphomas and are characterized by the expression of class-switched immunoglobulins with dense infiltration of reactive T cells and clusters or aggregates of CD123+ plasmocytoid dendritic cells.

494 BerEp4, Cytokeratin 17, and Cytokeratin 14 Immunohistochemical Staining Aid in Differentiatation of Basaloid Squamous Cell Carcinoma from Basal Cell Carcinoma with Squamous Features

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Background: Basaloid squamous cell carcinoma (BSCC) is an uncommon variant of squamous cell carcinoma which may histologically overlap with basal cell carcinoma (BCC) with squamous metaplasia. Differentiating between these neoplasms is important because of the worse prognosis associated with BSCC and the importance in determining the nature of basaloid metastases of unknown primary. BerEp4 immunohistochemical

staining can help distinguish conventional primary cutaneous BCC and BSCC, but is of limited utility in BCC with squamous differentiation, prompting the need to identify additional biomarkers to help distinguish these entities.
Design: We investigated BerEp4, Cytokeratin 17 (CK17) and Cytokeratin 14 (CK14) immunohistochemical staining in basaloid squamous cell carcinoma (n=15, with 5 cutaneous and 10 aerodigestive tract BSCC, including 3 lymph node metastases) and cutaneous basal cell carcinoma with squamous features (n=15). The percentage of tumor cells staining with these immunohistochemical markers was scored as 0 (none), 1 (<10% focal), 2 (10-80% patchy) and 3 (>80%diffuse positivity).
Results: The mean percentage of staining for all markers was significantly higher in the BCC with squamous differentiation group compared to the BSCC group (P=0.002 for BerEp4, P=0.0001 for CK17, P=0.0001 for CK14; unpaired t test). 80% (12/15) BCC displayed diffuse staining for all markers; 0% (0/15) of BSCC cases had diffuse staining for all three markers. While diffuse BerEp4 staining was observed in 3 BSCC cases, only focal or patchy/peripheral rim staining for CK14 and CK17 was present. The pattern of staining and % of positive cells are important to evaluate, rather than scoring as "positive" or "negative". To test the utility of this protocol, three additional cases of basaloid carcinoma of unknown primary metastatic to neck lymph nodes were stained. These tumors stained similarly to the basaloid squamous cell carcinomas with lack of diffuse positivity for all three markers. Five cases of p16 positive mucosal (anal canal) BSCC's showed a score or 0 or 1 for BerEp4 and 2 or 3 for CK14 and CK17 ($p<0.001$).
Conclusions: An immunohistochemical panel composed of BerEp4, CK14 and CK17 is useful for differentiating primary cutaneous BCC and metastatic basaloid-type tumors of the oropharynx and anus. This panel can be employed to better classify basaloid tumors with an aim towards guiding appropriate patient management.

495 Expression of Lymphatic-Specific Markers in Vascular Malformations

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Background: Vascular malformations (VM) are a heterogenous group of localized defects in vascular morphogenesis, composed of varying degree of lymphatic and/or vascular endothelium. Distinguishing lymphatic from vascular endothelial cells on routine histology can be very challenging.
Design: We sought to identify the degree of lymphatic and vascular components of VMs (n=32) by immunostaining for Prox-1, Podoplanin (D2-40), LYVE-1 and CD31. The test cases included paraffin-embedded tissues sections from lesions with lymphatic morphology: microcystic lymphatic malformation (n=7), mixed micro and macrocystic lymphatic malformation (n=7) and venous lymphatic malformation (n=10); and lesions with vascular morphology: venous capillary malformation (n=2) and Port-wine stains (n=6). were analyzed. Based on the degree of marker expression by the lesional cells, a semi-quantitative immunoscore was assigned as follows: 0, no expression; 1, <10%; 2, 11-50%; and 3, >51%.
Results: Lesions with lymphatic morphology consistently expressed both lymphatic and vascular markers whereas those with vascular morphology were negative for these lymphatic markers.
Conclusions: Immunostaining for lymphatic and vascular markers can be helpful in determining the degree of lymphatic and vascular endothelium in difficult to diagnose cases of VMs.

496 Pathological and Clinical Characteristics of Mammary Paget Disease: 25-Year Experience from a Major Tertiary Referral Center

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Background: Mammary Paget disease (MPD) is a rare entity. Previous reports have been limited by the small numbers of patients included. This retrospective study was designed to evaluate the clinicopathologic features of MPD and the outcomes of patients diagnosed with MPD at our institution over a 25-year period.
Design: We retrospectively examined MPD cases in our institution from 5/3/85 to 1/13/10. Available pathological characteristics, clinical presentation, radiological characteristics and outcome data were recorded.
Results: We identified 322 patients with MPD; 16 (5%) Paget without underlying breast carcinoma (PD), 97 (30%) Paget with DCIS (PDCIS) and 204 (63%) Paget with invasive ductal carcinoma (PIDC). Five MPD cases associated with lobular carcinoma were excluded. The median age at diagnosis of MPD was 52 years (range, 24 to 90). Skin changes were present in 92% (11/12) PD cases, 69% (46/67) PDCIS and 44% (61/139) PIDC ($p<0.0001$). Palpable tumor and abnormal mammograms were more likely to be found in PIDC ($p<0.0001$). Hormone receptor status was positive in 46% (20/44) PDCIS and 53% (81/153) PIDC tested. More than half of MPD-associated tumors were HER2-positive, 79% (19/24) and 61% (68/112) for PDCIS and PIDC, respectively. HER2 status was available for both Paget cells and underlying IDC or DCIS in 25 cases. All HER2-positive PDCIS and PIDC were associated with HER2-positive Paget cells (13/13). In contrast, HER2-negative PDCIS and PIDC were associated with HER-2 negative Paget cells in 67% (8/12) of cases tested and with HER2-positive Paget cells in 33% (4/12) of cases tested. Outcome data showed no locoregional or distant breast cancer recurrences in the fully excised PD group after 15 years of follow-up. There were more locoregional ($p=0.004$) and distant recurrences ($p<0.0001$) in patients with PIDC as compared to PDCIS.
Conclusions: Most MPDs were associated with an underlying cancer. PDCIS and PIDC are heterogenous tumors with regard to hormonal and HER2 status. HER2-positive IDC was associated with HER2-positive Paget cells in all cases with HER2 testing of both lesions available. MPD without an underlying carcinoma was rare and was not associated with recurrence, provided the lesion was completely excised. The three major groups of MPD have different clinicopathologic characteristics and survival rates, suggesting that these features can be incorporated into the management of MPD. Future work will compare PDCIS and PIDC to DCIS and IDC, adjusting for stage and treatment.

497 Expression of DICER, BIM, and PTEN in Malignant Melanoma: Role in Survival Mechanism and Adjuncts in Risk Stratification

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Background: Malignant melanomas (MM) are notoriously chemoresistant. The MITF-DICER-BIM and PTEN-BIM pathways are proposed to confer survival advantage in aggressive melanomas. MITF, the master regulator of melanocyte survival, upmodulates DICER, a critical microRNA processor. BIM, an inducer of apoptosis, is abrogated in the presence of DICER but upregulated by PTEN. *In vitro*, massive apoptosis occurs when BIM is overexpressed in the absence of DICER, whereas BIM suppression in the absence of PTEN confers resistance to BRAF inhibitors. The present study investigates whether these pathways are operative in clinical MM specimens and whether immunohistochemical (IHC) staining for these proteins is associated with metastatic status.
Design: Ten cases of nevi and twenty cases of MM (ten with and ten without metastasis) were identified from our institution's pathology archive (2006-2011). Cases were excluded if there was no sentinel lymph node biopsy or if the paraffin block was unavailable or contained insufficient tissue. Four-µm-thick sections of each lesion were immunolabeled for DICER, BIM, and PTEN. Staining intensity was scored as: nil, moderate (1+), or strong (2+).
Results: Nevi showed nil BIM expression but moderate (1+) staining with DICER and diffusely uniform strong (2+) staining with PTEN. BIM and DICER expression was increased compared to nevi in 55% and 45% of MM cases (n=20), respectively. Increased BIM and DICER expression was not associated with metastatic disease status. Contrary to the MITF-DICER-BIM model, BIM expression appeared unaffected by DICER status. However, BIM expression appeared directly correlated with PTEN in 60% of cases, suggesting multiple levels of regulation for BIM *in vivo*. Finally, PTEN expression was reduced in 55% of MM cases. The majority of these cases had metastasis (73%). Conversely, strong PTEN staining was more frequently seen in localized disease (78%).
Conclusions: Mechanisms of MM survival are complex and likely to involve multiple pathways not always evident in *in vitro* models. Although limited by a small sample size and heterogeneous sample subtypes, our results suggest that immunohistochemical staining for PTEN in primary MM holds promise as a useful adjunct for prognostication and risk stratification. Larger studies with long-term follow-up are warranted to confirm these findings and identify downstream PTEN targets for possible therapeutic application.

498 Presence of *K-ras* Mutations and Distinct Age-Associated Variations in Pilomatricoma

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Background: Previous reports suggested a bimodal age distribution and 4 morphologic developmental stages in pilomatricomas. *CTNNB1* mutations have been suggested as a causative event, but up to 39% of cases may not have this mutation. The association of β-catenin immunohistochemistry (IHC) with mutation status is controversial. We performed a comparative morphologic analysis in various age groups, assessed β-catenin IHC patterns, and examined *CTNNB1* and *K-ras* mutations.
Design: Clinical data, H&E slides, β-catenin IHC, *CTNNB1* and *K-ras* mutation status were assessed. A logistic regression analysis was performed to assess any association between variables.
Results: 611 cases were included (male: 51%; mean age 29.4 yrs, range: 1-87 yrs; mean size: 13 mm). A trimodal age distribution was observed with peaks in the 1st, 4th, and 7th decades. Head and neck was the most common site, followed by the extremities and trunk. H&E slides on 142 cases were evaluated for four previously described developmental patterns: proliferative, fully developed, early regressive (38%; most common) and late regressive. The presence of the following morphologic variables was significantly lower in the older patients: histiocytic reaction, myxoid changes, ossification, cartilage, calcification, and melanin pigment *($p<0.05$)*. Only 8% of examined cases (n=82) had a predominantly nuclear pattern for β-catenin IHC while most cases had either predominantly cytoplasmic (47%) or membranous staining (45%). There was no correlation between β-catenin IHC pattern and clinical data, morphology, or mutation status. Only 41% of tested cases (n=34) harbored *CTNNB1* mutation, but of cases tested for *K-ras* mutations (n=48), 35% were positive. The patients with β-catenin mutation were relatively younger (mean age: 24.7 yrs) compared to patients with *K-ras* mutation (mean age: 36.4 yrs).
Conclusions: There are significant morphologic variations between age groups in pilomatricomas. β-catenin IHC pattern is more frequently cytoplasmic/membranous rather than nuclear, suggesting involvement in cell-cell adhesion rather than the nuclear *Wnt* pathway of tumorigenesis. *K-ras* mutations are reported for the first time in pilomatricomas and they may play a distinct role in the development of this tumor.

499 Detection of Merkel Cell Polyomavirus in Formalin-Fixed and Paraffin-Embedded Tissues by Fluorescence *In Situ* Hybridisation and Its Correlation with qPCR

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Background: Merkel cell polyoma virus (MCPyV) is detected in 80% of Merkel cell carcinomas (MCC). The clonal integration and tumor specific mutations in the large T Antigen (LTAg) gene strongly implicate an oncogenic impact of the virus. To date the relationship between the viral presence and cancer induction, development or clinical prognosis is discussed controversial. Yet almost all studies are based on quantitative virus detection e.g. PCR or qPCR.

Design: In order to gain additional information about the quality of the viral presence on the single cell level we performed FISH analysis of formalin fixed and paraffin embedded (FFPE) MCCs (n= 62) on tissue micro arrays (TMA), determined the FISH pattern and correlated the results of the FISH analysis with the qPCR data. We grouped the MCCs according to different FISH evaluations and correlated them with the respective qPCR data on the basis of a determined cut-off. MCPyV FISH was established using the MKL-1 cell line which harbors integrated copies of MCPyV DNA. For MCPyV-qPCR the LT3 primer pair was used on whole tissue sections.

Results: MCPyV-FISH on FFPE MKL-1 cells revealed punctate signals compatible with viral integration. The MCPyV FISH positive MCC cores (76%) mainly revealed two different signal patterns: a punctate pattern (65%) which correlated with a moderate relative viral presence and in some areas the punctate pattern was combined with a diffuse pattern (11%) indicating the episomal presence of the virus which is linked to viral replication. If a pattern mixture was seen this was associated with very high qPCR values. Comparing MCPyV FISH and qPCR data the results correlated highly with 83 % in the MCPyV positive evaluated group, whereas the negative group showed a concordance of 93 %. The mean of the qPCR values of all MCPyV positive cores differed significantly from the negative cores (p= 0.0076). The FISH signals were in some tumor areas from the same patient heterogenic in intensity, pattern and localization.

Conclusions: While the qPCR using the LT3 primer pair was highly sensitive to detect MCPyV sequences in the respective MCC cases, the MCPyV FISH analysis highly concordantly revealed the quality of the viral presence, i.e. viral integration or episomal presence and morphological sublocalization in the tissues.

500 Comparison of Histopathology and Gene Expression Microarray Signatures of Spitzoid Tumors

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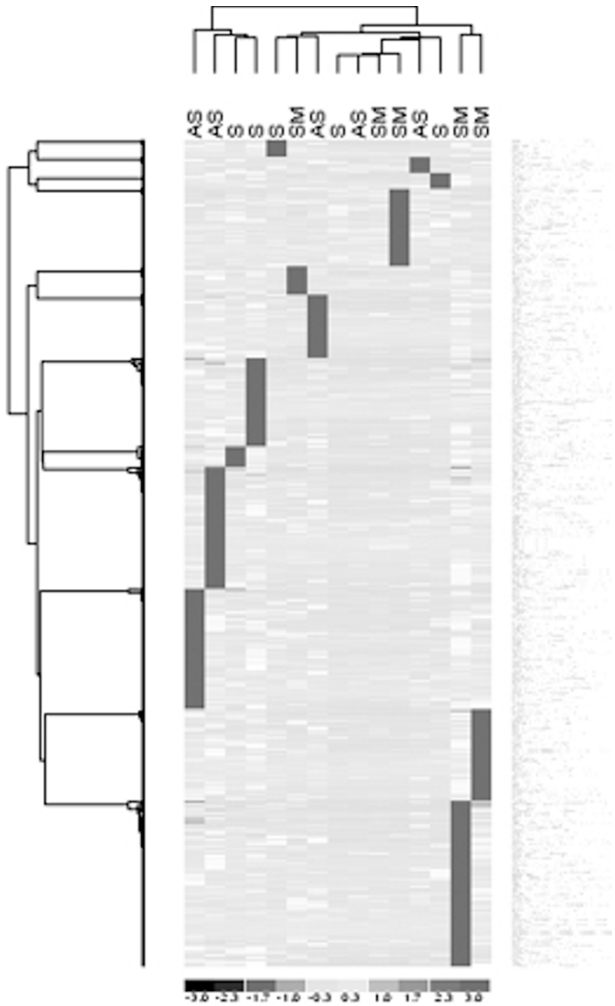
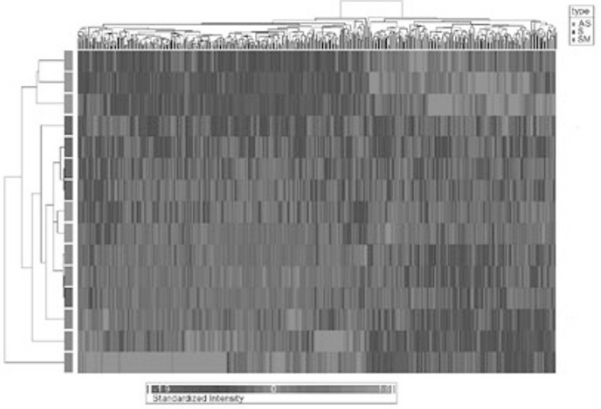
Background: Spitzoid tumors are often classified as Spitz nevus/tumor (S), atypical Spitz tumor (AS), or Spitzoid melanoma (SM) based on histopathologic appearance. We attempted to determine if gene expression microarray signatures (GAMS) derived from formalin-fixed, paraffin-embedded (FFPE) lesions diagnosed as S, AS, and SM correlate with histopathologic appearance.

Design: 15 FFPE samples, including 5 S, 5 AS, and 5 SM, were reviewed by 3 pathologists, cut into 8 µm sections, and dissected by LCM. Total mRNA was labeled with Nu-GEN WT-Ovation FFPE RNA Amplification System and FL-Ovation cDNA Biotin Module V2 kit, hybridized to the Affymetrix U133 Plus 2.0 Array. Differentially expressed genes were selected at a 1.5-fold expression difference and p<5.00E-2. Supervised clustering was performed with Partek Genomics Suite v.6.4, and unsupervised clustering was generated with dChip. Validation is in process.

Results: Unsupervised hierarchical cluster analysis failed to show clear clustering of AS, S and SM: the majority of samples (9 of 15) show no commonality of gene expression pattern correlated with their phenotype.



The supervised cluster was not able to yield unequivocally clear gene expression patterns for S, AS, and SM. 3 AS at the top of the cluster, 4 S just below, and SM at the bottom showed different gene expression profiles; however, 1 S fell into SM territory, and 2 AS clustered together with 2 S and 2 SM in the middle. Overall, SM tends to cluster together with a unique gene expression pattern apparently different from S and AS.



Conclusions: This study suggests that it may be possible to diagnose at least some SM as well as some lesions which would otherwise be diagnosed either S or AS as SM using a combination of histopathology and GAMS. Further study and clinical follow-up are necessary to confirm differences in gene expression and clinical behavior between SM and non-SM Spitzoid lesions.

501 Atypical Fibroxanthoma: Immunophenotypic Lineage Determination and Diagnostic Perspective

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Background: Atypical fibroxanthoma (AFX), typically a plaque/nodule on sun damaged skin, is considered a diagnosis of exclusion, yet requires strict histologic diagnostic criteria. Although it has a histologically aggressive appearance, it remains usually localized to the dermis, with limited extension into subcutis, and is usually cured by

complete excision. Currently, there are no reliable immunohistochemical markers to confirm the diagnosis of AFX. Our goal was to determine the utility of smooth muscle actin (SMA), CD271 (p75 NGFR) (a mesenchymal stem cell marker) and SOX-2 in the diagnosis of AFX.

Design: Eleven cases with the diagnosis of atypical fibroxanthomas were collected from our institution's pathology archives between the years of 2006 and 2011. Immunohistochemistry for smooth muscle actin, CD68, SOX-2 and CD271 (p75 NGFR) was performed on formalin-fixed paraffin-embedded archival tissue blocks.

Results: The histopathologic diagnosis was rendered by or verified in consultation with a certified dermatopathologist, and immunohistochemical slides stained and interpreted for SMA, CD271 (p75 NGFR) and SOX-2. Six (55%) of the cases of AFX showed immunopositivity for smooth muscle actin in a varying proportion of tumor cells, including pleomorphic cells, consistent with myofibroblastic/myogenic immunophenotype, given the absence of any hemangiopericytomatous pattern. All eleven (100%) cases expressed CD68 in a significant subset of tumor cells. CD271 showed cytoplasmic with/without membranous staining of any neoplastic cells in 8/11 (72%) cases, of which a significant proportion of cells was positive in 3/11 (27%). However, SOX-2 was uniformly negative in all cases with appropriate internal control. Additional immunostains found to be positive at the time of diagnosis included CD10 (5/5), vimentin (2/2), and factor VIIIA (1/2). Stains that were negative included pan-keratin (9/9), pan-melanoma (5/5), melan-A (2/2), S-100 (9/9), CD34 (3/3), high molecular weight keratin (1/1), CK5/6 (2/2), lysozyme (1/1), CD45 (1/1), and CD30 (1/1).

Conclusions: About half of AFX cases exhibit focal or diffuse myofibroblastic/myogenic immunophenotype, which in the context of CD271 positivity in at least a subset of tumor cells, in the absence of SOX-2 expression, suggests derivation of this neoplasm from mesenchyme-committed stem cells with secondary myofibroblastic/myogenic differentiation.

502 Study of the Transferrin Receptor Expression in Melanocytic Lesions: Diagnosis and Prognosis Evaluation

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Background: Melanoma is a malignant tumor with poor prognosis and is associated with a very high mortality rate. Treatment is limited and not very efficient even though new targeted therapies (anti-BRAF) are promising. The identification of molecules acting on cell growth could be another way to counteract tumor aggressivity. Highly dividing cells overexpress the transferrin receptor (TfR or CD71) as they require large amount of iron for DNA replication. Recent studies have also shown that cancer cells overexpress this receptor (TfR). The aims of our study were to analyze CD71 expression on benign and malignant melanocytic lesions in order to determine its diagnosis and prognosis value.

Design: CD71 immunohistochemistry was performed on 68 melanocytic lesions diagnosed at Rouen University Hospital between 2008 and 2011. The lesions included 30 benign melanocytic lesions (compound nevus, congenital nevus, blue nevus, Reed nevus, Spitz nevus and dysplastic nevus), 28 malignant melanocytic lesions (in situ, SSM, nodular, acral lentiginous, desmoplastic and spitzoid melanoma) and 10 melanoma metastases.

Results: Benign lesions disclosed no CD71 immunostaining, whereas 46.4% of melanomas were CD71+ with 50% for SSM, 60% for acral lentiginous melanomas and 83% for nodular melanomas. There was a 100% specificity for malignant lesions. CD71 staining seemed to be correlated to Breslow>2mm (p=0.0271). 90% of metastases were CD71+.

Conclusions: Our results show that this marker could be useful in the diagnosis of melanoma with 100% specificity. Its expression also seems to be associated with bad prognosis tumors and therefore could be used as a target to develop new drugs to improve patient treatment, especially in metastatic cases.

503 Use of Gene Expression Microarray To Discriminate Conventional Melanoma, Nevoid Melanoma and Benign Atypical Nevi

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Background: Nevoid melanoma (NM) is a form of malignant melanoma (MM) which mimics atypical nevi (AN). Histological features of NM are subtle and there is a high risk of misdiagnosis and delay of treatment. No molecular tests currently differentiate AN from NM. We sought to distinguish NM from AN and MM using gene microarray technology.

Design: Tissue from 8 MM, 7 NM and 10 AN was cut into 8 µm sections and dissected by LCM. Total mRNA was labeled with Nu-GEN WT-Ovation FFPE RNA Amplification System and FL-Ovation cDNA Biotin Module V2 kit, hybridized to the Affymetrix U133 Plus 2.0 Array. Differentially expressed genes were selected at 2-fold expression difference and FDR p<1.00E-2. Clustering and PCA were performed with Partek Genomics Suite v.6.4. Selected genes were validated by qRT PCR.

Results: Clustering analysis compared MM to AN and showed three distinct gene expression patterns for MM, NM and AN (Fig 1). Principle component analysis (PCA) of the 504 differentially expressed genes showed 3 phenotypes into 3 groups in 3 dimensional space that correlated with histopathology. One AN gene expression profile lay in NM territory (Fig 2).

In normalized gene signal intensity, gene PXT1 was more highly expressed in NM, as were HIF3a in AN and cREM in MM, compared to the other 2 groups respectively.

Fig. 1: Clustering Analysis

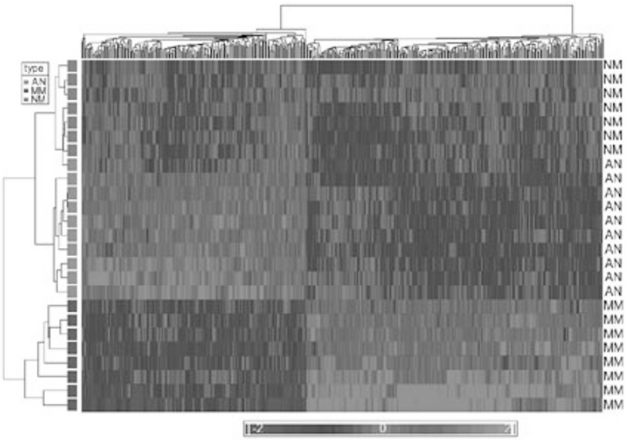
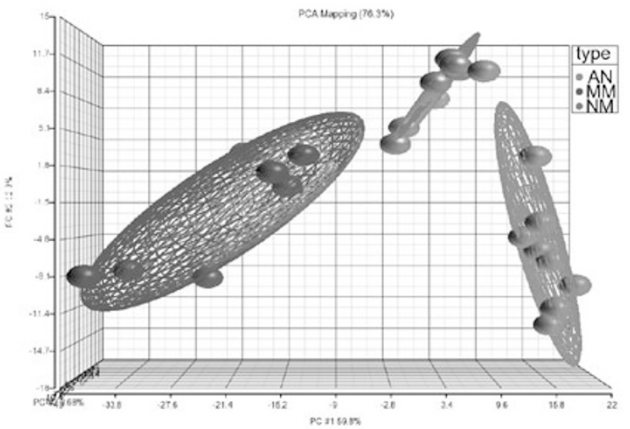


Fig. 2: PCA



Down-regulated FOXO3 and up-regulated CTLA4, PTPLA and MCHR1 genes in NM vs. AN were validated by qRT-PCR with average-fold changes of 0.64, 75.80, 2.71 and 45.31, respectively. Validation studies are on-going.

Conclusions: These results offer leads for novel molecular tests. Although commonality exists between NM and MM the overall discrete clustering of genes expressed in NM compared to AN and MM suggests a distinct molecular pathobiology for NM. Further study of genes expressed in NM may lead to novel tests which may distinguish these diagnostically challenging lesions.

504 Plexiform Spindle Cell Nevus: A Clinicopathologic Study of 122 Cases

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Background: Since its description, the proponents of plexiform spindle cell nevus (PlexiSCN) have emphasized its characteristic plexiform/fascicular architecture and predominant spindle cell phenotype. The authors have recently observed that angiotropism may further aid in the recognition and distinction of PlexiSCN from other melanocytic tumors.

Design: In this study, 122 PlexiSCN were evaluated for 21 clinical and histological parameters including angiotropism and/or neurotropism. Angiotropism and neurotropism were defined as melanocytes aligned along the external surfaces of blood vessels or nerves at the peripheries of the lesion. Additional tumor characteristics recorded for each lesion were: maximal diameter of the lesion (mm), symmetry, ulceration, circumscription, maturation, nodule formation, Breslow thickness (mm), Clark level, cell type, nevus/tumor components, configuration, mitotic rate (per mm2), presence of deep mitosis, significant cytologic atypia, recurrence or regrowth of tumor and positive margins.

Results: We found that the typical PlexiSCN architecture consisted of a symmetric, well-circumscribed plexiform/fascicular dermal melanocytic proliferation associated with angiotropism. PlexiSCN demonstrated a predominant spindled melanocyte phenotype. There were varying proportions of admixed epithelioid melanocytes, and the degree of melanocytic pigmentation differed slightly in each case. Melanocyte maturation was minimal; slight cytological atypia was characteristic. The mitotic rate was 0-2 per mm^2 and deep dermal mitoses were rare. Angiotropism was present in 100% of cases and neurotropism was present in 46.7% (57/122). The majority of PlexiSCN were associated with a conventional nevus (55.5% (57/122)); 11.5% (14/122) occurred with a blue nevus; 4.1% (5/112) occurred with a Spitz nevus. 37.7% (46/112) of PlexiSCN were not associated with a secondary melanocytic neoplasm. Ten lesions fell outside the typical PlexiSCN morphology and were "unclassifiable." One case was categorized as "atypical PlexiSCN." We are not aware of any adverse outcomes from our reported cases but detailed outcome data will be reported.

Conclusions: By employing the strict criteria of plexiform/fascicular dermal configuration, angiotropism, predominant spindle cell phenotype, and absence of Spitz and blue nevi characteristics, the plexiform spindle cell nevus can be reliably recognized as a distinct entity.

505 Sentinel Lymph Node Metastases in Problematic Spitzoid Melanocytic Tumors: Not a Predictor of Malignancy

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Background: The diagnosis and classification of spitzoid melanocytic tumors with atypical features remains problematic and controversial. Previously, sentinel lymph node mapping was advocated as a diagnostic test in this setting to potentially discriminate melanoma from benign tumors. Recent studies suggest that despite the presence of lymph node metastases, these patients almost always fare well. Here, we examined the outcome of patients with atypical Spitz tumors and spitzoid melanoma who received sentinel lymph node mapping, combined these findings with others' data and clarified the current state of the art in managing patients with these diagnostically challenging tumors.

Design: A search of the electronic files of the MGH Pathology Service identified forty-one patients treated with sentinel lymph node biopsy for atypical Spitz tumor or spitzoid melanoma from 1998-2008. These patients included 23 patients with atypical Spitz tumors and 18 patients with spitzoid melanoma. The following were recorded for each case: age, gender, location, status of the sentinel lymph node biopsy, status of the complete therapeutic lymph node dissection, treatment with interferon-alpha, duration of clinical follow up, recurrence or metastatsis beyond the regional lymph node basin, and death from metastatic disease.

Results: Sentinel lymph nodes were positive in 26% of atypical Spitz tumors (6/23) and 33% of spitzoid melanomas (6/18). One patient with Spitzoid melanoma developed in transit metastasis; none of the 41 patients developed metastases beyond the regional lymph node basin with a median follow up of 55 months. Follow up exceeded 4 years in 26 of 41.

Conclusions: SLN biopsy does not appear helpful as a discriminatory test or have a therapeutic benefit in patients with atypical Spitz tumors. Patients with spitzoid melanomas also appear to have a more indolent course than would be predicted by the presence of positive sentinel lymph nodes.

506 Impact of TRG@ Clonality Studies on the Diagnosis of T Cell Lesions

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Background: The diagnosis of cutaneous T cell lesions can be histologically challenging thus adjunct studies including: immunohistochemistry, flow cytometry, and T cell receptor gamma gene (TRG@) rearrangement studies are required. TRG@ studies can also be performed on peripheral blood (PB) for staging or to monitor disease status. The aim of this study was to evaluate the impact of TRG@ studies on the clinical diagnosis of T cell lesion.

Design: Institutional review board approval was obtained and medical records of patients with cutaneous T cell lesions (skin and/or PB) referred for TRG@ studies during 2004-2005 were reviewed. Study variables included: pathologic diagnosis of initial skin biopsy and frequency of TRG@ rearrangements (performed in house or at reference labs), frequency of PB testing, initial clinical diagnosis and final established clinical diagnosis on follow up.

Results: 91 cases were retrieved. 64/91 (70%) cases had a definitive histo-pathologic diagnosis on the initial skin biopsy. 26/64 (41%) had TRG@ assays ordered with concurrent results observed in 22/26 (85%), discordant results in 2/26 (8%) and inconclusive results in 2/26 (8%). PB involvement was determined by TRG@ assays in 15/40 (37.5%) cases as compared to 1 diagnostic case by flow cytometry and 2 diagnostic cases by Sezary preparations. Flow cytometry detected an atypical population of cells in 2 cases but these did not meet criteria for Sezary syndrome. 27/91 (30%) cases had an inconclusive initial histo-pathologic diagnosis and TRG@ studies had been ordered in all cases. One case had insufficient material for TRG@ studies and 7/26 (27%) cases had discordant histologic and TRG@ findings including 4 early mycosis fungoides (MF); 2 dermatitis cases and 1 case initially reported as MF which was subsequently determined likely to be a natural killer cell lymphoma.

Conclusions: Histology is superior to TRG@ assays in the diagnosis of skin lesions but TRG@ assays are more sensitive in detecting clones for PB involvement as compared to Sezary preparations and flow-cytometry. TRG@ assays provide important diagnostic information and are most useful when the pathologic diagnosis is inconclusive with 64% of these cases relying on these results to aid in the diagnosis. Further, the evaluation of T cell lesions is complex and the most accurate clinical diagnosis can be achieved when TRG@ rearrangement studies, histomorphologic findings and clinical information are simultaneously considered.

507 Clinicopathologic Features of Cutaneous Syncytial Myoepithelioma

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Background: Cutaneous myoepithelial tumors demonstrate heterogeneous morphologic and immunophenotypic features. We previously described a subset of cutaneous myoepitheliomas showing solid syncytial growth of ovoid, spindled or histiocytoid cells. We now present the clinicopathologic features of 36 cases of the distinctive syncytial variant of cutaneous myoepithelioma.

Design: 36 cases identified between 1997 and 2011 were retrieved from surgical and consultation files. 6 cases have been published previously. H&E and immunohistochemical stains were examined. Clinical and follow-up information was obtained from referring pathologists.

Results: Patients were 25 men and 11 women, with a median age of 36 years (range 2 months to 74 years). Anatomic sites were upper extremity (11), upper limb girdle (3), lower extremity (13), back (5), face (2), chest (1), and buttock (1). Lesions ranged in size from 0.3 to 2.7 cm (median 0.7 cm) and were well circumscribed and either polypoid or papular. All tumors were centered in the dermis. Microscopically all tumors showed a solid sheet-like growth of uniformly sized ovoid to spindled or histiocytoid cells with pale eosinophilic syncytial cytoplasm. Nuclei were vesicular with fine chromatin and small or inconspicuous nucleoli, and showed mild to no atypia. Mitoses ranged from 0 to 4 per 10 HPF; 26 tumors showed no mitoses. All tumors lacked necrosis and lymphovascular invasion. Adipocytic metaplasia, appearing as superficial fat entrapped within the tumor, was seen in 9 cases. Chondro-osseous differentiation was seen in 1 tumor. All tumors examined were diffusely positive for EMA and S-100 protein. Keratin staining was focal in 1 of 33 tumors and seen in rare cells in 2 other cases. There was also positivity for GFAP (14/31), SMA (9/13), and p63 (6/11). Most patients were treated by local excision. Follow-up information is available for 19 patients so far (mean follow-up 16 months); all are alive with no evidence of disease, including 7 patients who had positive deep margins. None has recurred to date.

Conclusions: Cutaneous syncytial myoepithelioma is a distinct variant that more frequently affects men, occurs over a wide age range, and usually presents on the extremities. Tumors are positive for S-100 and EMA, and unlike most myoepithelial neoplasms, keratin staining is infrequent. Prior reports describe some risk of recurrence and metastasis for cutaneous myoepithelial tumors; however, based on preliminary follow-up data, the syncytial variant appears to behave in a benign fashion.

508 Nevi with Epithelioid Cytomorphology and Architectural Disorder

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Background: Dysplastic nevi (dysN) were recognized over 30 years ago as melanocytic proliferations with distinctive architectural and cytologic features in families with relatively high incidence of melanoma. Numerous descriptions of dysN have been published; however nevi with epithelioid cytologic features and architectural disorder (NACED) have not been as well described.

Design: To further understand the clinical and histologic characteristics of NACED, we reviewed all 40 identified cases of NACED diagnosed at our institution from 1997 to 2010. Medical records were reviewed, and patients were separated into categories based on personal history of multiple dysN, personal history of malignant melanoma in situ and/or invasive melanoma, and family history of melanoma. Histologic sections were reviewed and 23 pathologic features were compared among the groups of patients.

Results: No single histologic feature statistically correlated with personal history of multiple dysN or melanoma, family history of melanoma, or lack of personal or family history. 89% of patients with a personal or family history of melanoma had lesions that were categorized to have overall high-grade morphology. Conversely, only 64% of patients without a personal or family history of melanoma had similar lesions (Table).

Histologic Features of Nevi with Epithelioid Cytomorphology and Architectural Disorder

Histologic Feature	All Patients (%)	No Personal or Family History of Melanoma (%)	Multiple DysN (%)	Personal History of MMIS or Melanoma (%)	Family History of Melanoma (%)	Personal or Family History of Melanoma (%)
Overall High Grade	75	64	64	86	100	89
High N/C Ratio	20	18	36	43	25	33
Large Nucleoli	55	41	55	71	50	67
Coarse Chromatin	38	41	46	43	50	33
Intraepidermal Mitoses	48	41	64	43	25	33
Kamino Bodies	35	36	46	29	50	33
Pagetoid Lateral Spread	50	41	18	14	25	22
Lack of Lateral Circumscription	68	67	73	86	50	78
Clefts Around Nests	25	23	9	29	0	22
Bridging of >3 Rete	18	82	82	14	50	67
Focal Atrophy	23	18	18	17	25	11
Dermal Mitoses	31	28	27	17	0	14
Lack of Dermal Maturation	80	22	18	71	0	14

Conclusions: In this largest series to date of NECAD, there was no correlation between a wide array of histologic features and personal or family history of melanoma. While high grade morphology was more frequent in patients with a personal or family history of melanoma, this could not be statistically validated with this small data set. These findings raise the possibility that these lesions may not have the same prognostic significance as traditional dysplastic nevi.

509 Predictors of BRAF Mutation in Melanocytic Nevi: Analysis across Regions with Different UV Radiation Exposure

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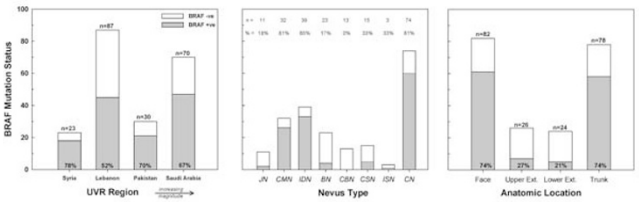
Background: BRAF mutations have been implicated in initiating pro-mutagenic cellular proliferation mostly based on homogeneous Western cohorts. Data addressing the possible interaction between exposure to different solar UV radiation (UVR) magnitudes and BRAF mutation rate (BMR) in Melanocytic Nevi (MN) are limited.

Design: Extended BRAF testing for 9 mutations in 225 MN cases derived from 215 patients from 4 different UVR regions: Lebanon (n=95; 110 kJ/m2/year), Syria (n=23; 93.5 kJ/m2/year), Saudi Arabia (n=70; 139kJ/m2/year) and Pakistan (n=37; 118kJ/

m2/year) was performed. Data collected included: age, gender, anatomic location and lesion size. Histologic parameters recorded were: MN type [junctional (JN), compound (CMN), intradermal (IDN), blue (BN), cellular blue (CBN), compound spitz (CSN) and intradermal spitz (ISN), congenital (CN)], solar elastosis grade and nevus pigmentation degree. Cumulative 21-year UVR averages were derived from The National Center for Atmospheric Research.

Results: BRAF mutation status was obtained in 210 cases (6.7% failed PCR). Overall BMR was 62.4% (131/210) with V600E mutation accounting for 98.5% of cases. Discordant mutation status was found in 2/10 patients with multiple nevi. BMR differed significantly, yet non-systematically, among UVR regions (fig.1 left). Mutation rates varied widely across MN type (fig.1 middle; p<0.001) and by anatomic location (fig.1 right; p<0.001). Severe pigmentation was less frequent in BRAF mutation positive nevi [5/131 (4%) vs. 34/79 (43%); p<0.001]. Multivariate independent predictors of BRAF mutation in MN were: age [OR (95% C.I.) = 1.43 (1.13-1.74) per 10 years; p=0.004], anatomic location [p=0.043 overall] and nevus type [p<0.001 overall]. UVR region was not an independent predictor of BRAF mutation.



Conclusions: Increased BRAF mutation with age along with the lack of a UVR magnitude - BRAF mutation association suggests that duration of exposure rather than UVR exposure dose is the more likely link to acquiring the mutation. Our results also point to a possible protective role of increased pigmentation against acquiring BRAF mutation.

510 Herpetic Dermatitis: Correlation of Clinical Impression, Histopathologic Findings and PCR

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Background: Herpetic dermatitis (HD) due to herpes simplex virus (HSV) and varicella zoster virus (VZV) can present with similar clinical and histologic features, although treatment for these two entities differ. Confounding matters, viral cytopathic changes are not always observed in biopsy specimens. Therefore, PCR can play an integral role in the definitive diagnosis of HD, and in the distinction of VZV from HSV-1/2. We correlated clinical impression, histologic findings and PCR results. We also describe cases of HD without viral change ("herpes incognito") that histologically demonstrate the pattern of a dermal hypersensitivity reaction (DHR).

Design: 40 patients with skin biopsies from NorthShore University HealthSystem (2004-2011) had PCR performed to detect HSV-1/2 or VZV. Patient demographics, clinical impression and histologic characteristics were reviewed and correlated with PCR findings.

Results: Patient demographics and results are illustrated in tables 1 and 2. Sites include head & neck, face, chest, abdomen, back, extremities, penis, and perineum.

Patient Demographics

Total Number of Patients	Mean Age	Female	Male
40	53 (range 10-90)	22 (55%)	18 (45%)

Results

Clinical Impression	Histologic Viral Changes	PCR Positive	HSV-1	HSV-2	VZV	HSV&VZV
Herpetic dermatitis (28)	Present (21/28)	21/21	8	2	11	0
	Absent (7/28)	3/7	1	1	1	0
Other (12)	Present (12/12)	12/12	7	1	2	2

Overall, there was discordance between the clinical impression and histologic findings in 19 cases (48%) and PCR confirmed HSV or VZV infection in 15 of these 19 cases. In PCR confirmed cases of herpes where the clinical impression was HD but histologic viral changes were absent, histopathologic findings demonstrated a superficial and deep perivascular mixed cell dermatitis with eosinophils (DHR).

Conclusions: 1.Studies have reported HD (mostly commonly secondary to VZV) without diagnostic viral cytopathic effect as "herpes incognito". These cases usually demonstrate a superficial and deep perivascular and perifollicular lymphocytic infiltrate without significant eosinophils. We observed similar findings in 3 cases (2HSV, 1 VZV) but unique to this study, our cases contained a prominent eosinophilic infiltrate, mimicking a DHR.

2.The results of this study suggest that routine use of PCR for definitive diagnosis of HD should be considered when there is a clinical suspicion of herpes virus infection, even when there is a lack of specific histopathologic findings.

3.DHR should be recognized as one histologic manifestation of herpes incognito.

511 Immunohistochemical Staining for p-ERK: A Potential Screening Tool for *BRAF* Mutations in Melanoma

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Background: BRAF V600E mutations are frequent in malignant melanoma (MM), resulting in constitutive ERK signaling and tumor cell proliferation. Documentation of BRAF exon 15 mutation in MM is required for treatment with the BRAF-mutant kinase inhibitor, vemurafenib. We hypothesized that phosphorylated ERK (pERK) immunohistochemical staining might distinguish BRAF wild type (WT) and mutated tumors and serve as a screening tool.

Design: pERK1/2$^{THR202/Tyr214}$ immunostains performed on 27 MM with known *BRAF* mutational status were scored as percentage of positive cells (0-100) and intensity of cytoplasmic and nuclear staining (1-3 for each). The summed products of percentage positive and cytoplasmic and nuclear staining intensity (total stain score) was tabulated. Mutations in *BRAF* exon 15 and *NRAS* exons 1 & 2 were determined by PCR and DNA sequencing from formalin-fixed paraffin embedded, microdissected tissue. Two tailed Mann-Whitney U test was employed.

Results: Of 27 MMs, 7 harbored a *BRAF* mutation: 6 (V600E) and 1 (K601N). Of 20 *BRAF* WT tumors, 5 were *NRAS* mutants (4xQ61K; 1xQ61R). 15/15 *BRAF* WT MM had a total stain score of ≤100 (mean+/-SEM=35+/-9.6, median= 40, range=0-100) and each showed pERK positivity in ≤ 25% of tumor cells. For *BRAF* mutated cases, 6/7 had a total stain score of ≥150 (mean+/-SEM=220+/-37, median=200, range=75-375). All had at least 25% of tumor cells staining, with 5/7 having ≥50% positivity (mean 52%). *NRAS* mutated cases were more heterogeneous but tended to have increased pERK over WT (mean+/-SEM=134+/-31, median=100, range=45-225). The pERK total stain scores between the *BRAF* mutated and WT groups was statistically significant (p<0.001). Using a cutoff of 25% cell positivity, the positive and negative predictive value of pERK immunostaining for *BRAF* mutation was 50% and 100%. *NRAS* mutated cases, which also activate the ERK pathway, lowered the positive predictive value.

Conclusions: In this cohort, use of the 25% cutoff would have identified all *BRAF* mutated cases, and reduced testing of ultimately negative cases from 20 tests to 7 tests (65% reduction). pERK immunostains may be useful as a screening tool for *BRAF* mutations and confirm this known signaling cascade in fixed specimens. Given the known time-dependent lability of phosphoproteins, we would recommend application only in rapidly fixed, small biopsy or cytologic samples.

512 Fully Automated Dual-Color Dual-Hapten Silver In-Situ Hybridization Staining for *MYC* Amplification: A Diagnostic Tool for Discriminating Secondary Angiosarcoma

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Background: *MYC* amplification has been shown to selectively occur in secondary cutaneous angiosarcoma. We have tested the ability of automated dual-color dual-hapten silver in-situ hybridization (DDISH) staining to correlate with fluorescence in-situ hybridization (FISH) for *MYC* amplification and to discriminate secondary angiosarcoma (SAS) from atypical vascular lesions (AVL).

Design: Cases of SAS (n=7) and AVL (n=3) were retrieved and examined by FISH and DDISH. DDISH staining was performed using the Dual Color Open Probe software on a Ventana Benchmark XT automated slide stainer. DNP labeled/repeat depleted *MYC* and DIGoxigenin labeled Chromosome 8 (CHR8) probes were combined into one Prep Kit Dispenser. Pretreatment conditions were: Extended deparaffinization at 72°C, 3 cycles of cell conditioning with CC2 for 12 minutes at 80°C, and protease digestion with ISH protease 3 for 32 minutes. Denaturation was at 80°C for 12 minutes; hybridization at 44°C for 6 hours was followed by stringency washes (3 at 72°C, 8 minutes). *MYC* DNP probe was detected with ultraView silver ISH (SISH) DNP Detection Kit silver anti-hapten antibody (20 minutes), and SISH detection (8 minutes). CHR8 DIG probe was detected with ultraView Red ISH DIG Detection Kit; anti-hapten antibody incubated (20 minutes) and Red detection (8 minutes). The slides were counterstained with hematoxylin II for 8 minutes, post counterstained with bluing reagent for 4 minutes, and mounted as permanent slides for bright field microscopy. Metallic black silver (*MYC)* and reference CHR8 red signals were qualitatively and semi-quantitatively enumerated for tumor nuclei. Small and large clusters of silver signals were recorded as 6 or 12 signals respectively. *MYC* amplification was defined as *MYC*/CHR8 ratio >2.0.

Results: Where tissue was available for both DDISH and FISH, all SAS cases demonstrated *MYC* amplification (7/7=100%) by both DDISH and FISH. All AVL were negative for *MYC* amplification by both techniques (0/3=0%).

Conclusions: In this cohort of cases, use of DDISH identified all *MYC* amplified cases, and distinguished SAS from AVL. These data suggest that DDISH staining may be useful in distinguishing SAS from AVL in challenging cases.

513 Pigmented Basal Cell Carcinoma: Increased Melanin or Increased Melanocytes?

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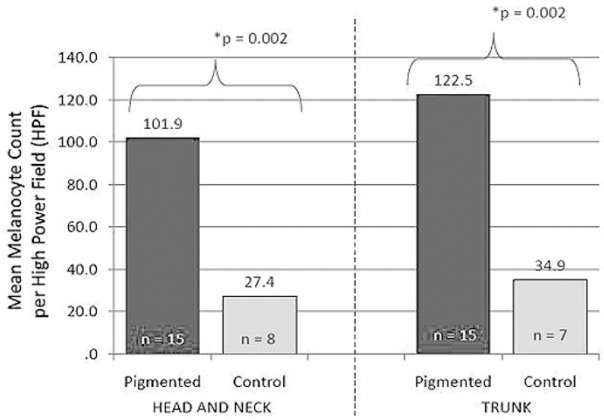
Background: Pigmented basal cell carcinoma (pBCC) often mimics melanoma clinically and occasionally histologically. Although infrequent, there can be collision tumors between pBCCs and melanocytic neoplasms. Melanocytic immunohistochemical stains such as Mart-1/Melan-A may be used in select cases. Therefore, we sought to determine whether pBCC actually contains more melanocytes than non-pigmented basal cell carcinoma (nBCC) or if pBCCs simply have a relative increase in melanin production.

Design: Under an IRB-approved protocol, fifteen pBCCs from the head and neck (sun-exposed) and fifteen pBCCs from the trunk (sun-protected) were obtained from our case files. In addition, fifteen nBCCs (8 from the head and neck, 7 from the trunk) were obtained as controls. All cases were reviewed by a board-certified dermatopathologist to confirm the historical diagnosis prior to inclusion. Patient age and gender were also recorded prior to de-identification. Mart-1/Melan-A staining was performed on cases and controls. An evaluator identified the area of greatest staining density on low-power then manually counted and recorded the number of positively staining cells in a single 400x field in this area.

Results: The mean count of melanocytes/400x HPF in pBCCs from the head and neck was 101.9 compared to 27.4 in nBCCs (p=0.002). The mean count of melanocytes/400x HPF in pBCCs from the trunk was 122.5 compared to 34.9 in nBCCs (p=0.002) (Figure 1).



Age and gender were comparable among the study cases and controls (Figure 2).

Pigmented BCC Samples		
Head/Neck	Mean Age (years):	64
	Male:Female	1:0.67
Trunk	Mean Age (years):	60
	Male:Female	1:1.14
Controls		
Head/Neck	Mean Age (years):	73
	Male:Female	1:0.60
Trunk	Mean Age (years):	66
	Male:Female	1:2.5

Conclusions: Our study concludes that pBCCs have an increased number of melanocytes as compared with nBCCs at site-matched locations. This information may aid in the interpretation of melanocytic immunohistochemical stains in pBCCs especially cases in which malignant melanoma is in the differential or a collision tumor is present.

514 Cutaneous Marginal Zone Lymphoma: A Multi-Institutional Clinicopathologic Study

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Background: It is unclear whether the immunophenotyic and morphologic features of primary cutaneous marginal zone lymphoma (CMZL) significantly differ from marginal zone lymphomas occurring in other sites. Recent studies suggest that a subset of pts are at risk of developing systemic disease.

Design: A search of the pathology files of 2 tertiary care institutions was performed. Clinicopathologic features of the identified cases were reviewed.

Results: 40 pts (73 biopsies) were identified.Mean age at diagnosis was 54 years (range 19-85y); M:F ratio was ~1.8:1. Pts presented with ≥1 lesions described as plaques, nodules, papules, or tumors, in the following sites: head and neck (18 biopsies), trunk (30 biopsies), upper extremities (14 biopsies), or lower extremities (5 biopsies); 5 pts presented with concomitant involvement of more than one body region. The most common growth patterns were nodular and diffuse, with sparing of the overlying epidermis. Cells were CD43+ (55% of cases), BCL2+ (82%), and CD10+ (3.5%), and were uniformly negative for BCL6 (29 cases) and CD5 (28 cases). CD21 highlighted follicular colonization in 12 tested cases, and highlighted the malignant cells in 1 case. IgH gene rearrangement studies were (+)in 6/12 (50%) of tested cases, and kappa/lambda immunoglobulin light chain ISH or IHC had a monoclonal pattern of reactivity in 23/29 (79%). 5 pts had antecedent biopsies that, although suspicious, were nondiagnostic for lymphoma. 1/8 tested pts had a positive staging bone marrow bx. Pts were treated with localized irradiation (2 cases), rituximab (5 cases), or intralesional steroids (5 cases); most pts (~70%) received no therapy besides excisional biopsy. 6 patients had recurrent disease, all in the same body region as at original presentation. Besides the pt with bone marrow involvement, no pts to date developed lymphoma outside the skin.

Conclusions: 1) A CD5-/CD10-/BCL6- phenotype was, with 1 exception, observed in all tested cases. CD21 aided identification of follicular colonization. 2) percentages of CD43 and BCL2 positive cases were similar to those observed in marginal zone lymphoma occurring in other sites. 3) Kappa/ lambda ISH and IHC were more sensitive than IgH gene rearrangement studies, likely due to technical problems associated with extraction of sufficient DNA for PCR studies. 4)CMZL has a low likelihood of localized recurrence and very rarely disseminates, perhaps justifying a conservative approach to clinical management when the pathologic diagnosis is certain.

515 Histopathologic Features of Mycosis Fungoides

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Background: Mycosis fungoides (MF) is a distinct clinicopathologic peripheral T-cell lymphoma. The histopathology of MF is not unique. In fact, its features can significantly overlap with benign dermatitis. Herein, we describe the histologic features of seventy-three cases of MF (two are folliculotropic) and compare those features to a similar number of cases spongiotic dermatitis and psoriasiform dermatitis.

Design: The Duke Pathology clinical database was searched for cases with the diagnosis of "mycosis fungoides". After the patients were identified, those with a concurrent, positive T-cell gene rearrangement study (TCRGR) were selected. Seventy-three biopsies met the predetermined inclusion criteria. Fifteen histologic parameters on each biopsy were evaluated including the following: parakeratosis, ulceration, dyskeratosis, civatte bodies (intraepidermal necrotic keratinocytes), spongiosis, Langerhan cell collections, interface dermatitis, fibroplasia, lichen simplex chronicus changes (LSC), mast cells, eosinophils, neutrophils, margination of neutrophils, colloid bodies (dermal) and dermal edema. Spongiosis was graded on a spectrum from absent to severe, while the cellular infiltrates were either absent, <10%, or >10%. A similar number of biopsies with the descriptive diagnosis of spongiotic dermatitis (SD) and psoriasiform dermatitis (PD) were analyzed using identical criteria. The histopathologic characteristics of the three groups were calculated to determine if distinct characteristics exist.

Results: There were several histologic features that were more or less common in MF than the other two groups. Langerhan cell collections were almost 10X more common in SD and PD than in MF (MF-1.4%, SD-12.3%, PD-18.4%). LSC changes were most characteristic of PD (MF-5.5%, SD-6.8% and PD-46.1%). Colloid bodies were present more than 10X more frequently in MF than in SD and PD (MF-19.2%, SD-1.4% and PD-1.3%). Neutrophils in the dermis were observed less frequently in MF (MF-18.9%, SD-54.8% and PD-50%). Approximately 50% of the MF cases in our study contained eosinophils. Interestingly spongiosis was present in 64.4% of MF biopsies, in contrast to SD-100% and PD-82.1%.

Conclusions: This series noted an association of certain histologic features and MF, namely: the presence of colloid bodies and the absence of Langerhan cell collections, neutrophils in the dermis and LSC changes favored a diagnosis of MF. Spongiosis, although less frequently observed in MF as compared to SD and PD, was still present in almost 2/3 of the cases in our series. Finally, almost 50% of the MF cases contained eosinophils.

516 Acral Nevi as Possible Precursors to Acral Melanomas?

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Background: Melanocytic nevi with site-related atypia display inherent architectural disorder and cytologic abnormalities that can mimic dysplastic nevi or melanoma. Under this category, these nevi include melanocytic proliferations from acral, genital, special site (breast, flexural, scalp and auricular), and conjunctival areas on the body. To date, it is general accepted that that nevi with site-related atypia are biologically stable and do not possess an increased risk of malignant transformation. However, it has been the experience of one of the authors (CMM) that acral melanoma can arise in the background of an acral nevi precursor lesion.

Design: To investigate this further, a natural language search within the division of dermatopathology case database at Weill Cornell Medical College was conducted from 2006 to 2011 to identify cases of acral melanoma. Melanomas occurring on the ankle, foot, hand, and nail apparatus were considered of true acral origin. Reports of cases of acral melanoma were reviewed to see if a precursor lesion was present.

Results: Our search yielded a total of 48 acral melanomas, of which 39 (81%) were of acral lentiginous type, 8 (17%) were of superficial spreading type, and 1 (2%) was of nodular type. Of the 48 patients, 32 were female and 16 were male. The age ranged from 30 years to 98 years, with an average age of 62. Within the acral lentiginous melanomas and superficial spreading melanomas, 10 of 41 (24%) and 5 of 9 (56%) arose from a precursor acral nevus respectively. These acral melanomas with precursor acral nevi all occurred on the foot with 6 of 15 lesions occurring on the plantar aspect, 8 of 15 lesions occurring on the dorsal aspect, and 1 case with an unspecified biopsy site. Interestingly, 6 of 48 (12%) acral melanomas arose from either nail matrix or nail bed; all of which did not show any evidence of a precursor lesion.

Conclusions: Our results challenge the commonly held viewpoint that acral melanomas rarely arise from a precursor lesion and more often arise de novo. We acknowledge that this may indeed be the case for acral melanomas of the nail matrix and nail bed. However, a diagnosis of acral nevus may in fact require closer clinical follow-up and more aggressive management. Further investigation into the molecular alterations of acral nevi are needed to correlate with known molecular alterations in acral melanomas such as mutations in the receptor tyrosine kinase KIT or cyclin D1 gene amplifications.

517 Lentiginous Compound Dysplastic Nevus of the Back – A Mimic of Recurrent Nevus and Malignant Melanoma with Regression

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Background: Melanocytic nevi of the back can have 'recurrent' features attributed to repeated trauma; however, these features have not been emphasized in the literature.

Design: Retrospective review of 1,015 consecutive lentiginous compound dysplastic nevi (LCDN) from a back location without prior biopsy between 1/2010-2/2011 yielded 54 cases with localized fibrosis. For histologic comparison, 58 consecutive cases of melanoma with regression diagnosed between 6/2004-8/2011 were included.

Results: The median age of LCDN patients was 43 (range 21-85). 27/54 (50%) and 27/54 (50%) LCDN cases with fibrosis had a "recurrent" and traumatic pattern, respectively. The median age of regressed melanoma patients was 63 (range 24-93) and

median Breslow depth was 0.61 mm (range 0.2-49). Junctional extension beyond the dermal fibrosis, adnexal involvement, effacement of the retiform epidermis, cytologic atypia, nests of melanocytes admixed with lymphoid infiltrate, elastosis below dermal fibrosis, prominent melanophages, and plasma cells were significantly more frequent in melanoma.

Table 1: Summary of histologic features.

	Regressing melanoma	LCDN	P value
Lentiginous junctional melanocytic proliferation	58/58 (100%)	50/54 (93%)	0.051
Confluent growth pattern	52/58 (90%)	22/54 (41%)	<0.0001*
Extension beyond dermal scar/fibrosis	58/58 (100%)	0/54 (0%)	<0.0001*
Adnexal involvement	50/58 (86%)	14/54 (26%)	<0.0001*
Effacement of the retiform epidermis	49/58 (84%)	28/54 (52%)	0.0002*
Pagetoid spread	52/58 (90%)	11/54 (20%)	0.0001*
Junctional mitotic figures	4/58 (7%)	1/54 (2%)	0.365
Cytologic atypia (prominent nucleoli, enlarged nucleus & cell size)	51/58 (88%)	15/54 (28%)	<0.0001*
Prominent cytoplasmic melanin pigment	25/58 (43%)	12/54 (22%)	0.026*
Nests of melanocytes admixed with lymphoid infiltrate	25/58 (43%)	2/54 (4%)	<0.0001*
Elastosis below dermal scar/fibrosis	11/58 (19%)	0/54 (0%)	0.0006*
Dermal nevus below dermal scar/ fibrosis	2/58 (3%)	49/54 (91%)	<0.0001*
Dermal inflammation#	40/58 (69%)	14/54 (26%)	<0.0001*
Plasma cells#	30/58 (52%)	2/54 (4%)	<0.0001*
Melanophages#	30/58 (52%)	2/54 (4%)	<0.0001*

* Statistically significant, two-tailed P-value <0.05, Fisher's Exact test; #Scored as focal/subtle or marked; remaining features scored as present or absent

A dermal nevus component below the dermal fibrosis was significantly more frequent in LCDN.

Conclusions: LCDN of back can have 'recurrent' features despite lack of prior biopsy. Recognition of these histologic features can be helpful in distinguishing it from severely atypical melanocytic proliferation and melanoma with regression.

518 Expression of CD10 and MMP-11 in the Differential Diagnosis of Dermatofibroma Variants and Dermatofibrosarcoma Protuberans

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Background: Dermatofibrosarcoma protuberans (DFSP) is a fibrohistiocytic tumor of intermediate malignancy and carries significant risk of local recurrence. The distinction between DFSP and dermatofibroma (DF), especially its variants (VDF), can be challenging. CD10 and MMP-11 were found to be useful markers for the differential diagnosis of classical DF and DFSP. We sought to characterize the staining patterns of CD10 and MMP-11 in a large set of VDF and study the utility of these two stains in differential diagnosis of VDF and DFSP.

Design: Immunohistochemical (IHC) stains were performed on 31 cases of DFSP, 38 cases of DF, and 41 cases of VDF using antibodies to CD34, Ki67, CD10, and MMP-11. Of 41 VDF cases, 18 were aneurismal type, 16 lipidized type, 4 with both aneurismal and lipidized features, 1 cellular type, 1 with monster cells, and 1 atypical fibrous histiocytoma.

Results: Thirty-six of 41 (88%) VDF cases and 37 of 38 (97%) DF cases were CD34 negative but only 2 of 31 (6%) DFSP cases showed no staining of CD34. All DFSP cases were positive for Ki67 and 22 of 31 (71%) cases had positive Ki67 stain in 5% or more tumor cells. Comparable staining patterns were observed in 5 of 41 (12%) VDF cases and 1 of 38 (3%) DF cases. CD10 and MMP-11 staining results were shown in Table 1.

Table1. CD10 and MMP-11 Expression in DFSP, DF, and VDF

	CD10			**MMP-11**		
IHC results (% of tumor cells positive)	**DF (38)**	**VDF (41)**	**DFSP (31)**	**DF (38)**	**VDF (41)**	**DFSP (31)**
0%	2.6% (1)	0% (0)	9.7% (3)	0% (0)	0% (0)	77.4% (24)
1 - 10%	0% (0)	0% (0)	29.0% (9)	2.6% (1)	34.2% (14)	16.1% (5)
10 - 25%	10.5% (4)	7.3% (3)	22.6% (7)	2.6% (1)	7.3% (3)	6.5% (2)
25 - 50%	23.7% (9)	17.1% (7)	19.4% (6)	10.5% (4)	26.8% (11)	0% (0)
51- 75%	36.8% (14)	41.5% (17)	12.9% (4)	31.6% (12)	12.2% (5)	0% (0)
76 -100%	23.7% (9)	34.1% (14)	0% (0)	50.0% (19)	19.5% (8)	0% (0)
Other*	2.6% (1)	0% (0)	6.4% (2)	2.6% (1)	0% (0)	0% (0)

* staining patterns can't be determined due to loss of tissue.

Of the 2 DFSP cases that were CD34 negative, both demonstrated over 50% Ki67 positivity and were MMP-11 negative as well. Of the 5 VDF cases that were CD34 positive, distinctions from DFSP were supported by strong positive staining of MMP-11 and CD10 and very low expression of Ki67.

Conclusions: Our results demonstrate that VDF has staining patterns comparable to classical DF. MMP-11 and CD10 stains can be useful in the differential diagnosis of VDF and DFSP.

519 Primary Cutaneous Rhabdomyosarcoma: A Clinicopathologic Review of 11 Cases

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Background: Rhabdomyosarcoma (RMS) is a sarcoma with skeletal myogenic differentiation that is most often found in the head and neck, genitourinary tract, or deep soft tissue. Primary cutaneous RMS is extremely rare with < 20 documented cases in the English literature, only 5 of which presented in adults.

Design: Cases diagnosed as RMS were retrieved from the archives and consultation files of 3 institutions. For inclusion, cases had to arise in the dermis/subcutis with no evidence of a metastatic origin. Cases were subclassified as embryonal, alveolar, pleomorphic, and not otherwise specified (NOS) RMS. Clinicopathologic features were retrospectively reviewed.

Results: 11 cases met selection criteria. The tumors occurred in a bimodal age distribution including 5 pediatric patients (4M;1F, mean age 10 yrs, range 0.25-17 yrs) and 6 adults (4M;2F, mean age 74 yrs, range 60-87 yrs). The lesions involved the head and neck (6 cases), the extremities (4 cases), and the trunk (1 case). The adult subset consisted of pleomorphic (4 cases) and NOS (2 cases) subtypes while the pediatric subset demonstrated alveolar (1 case), embryonal (2 cases), and NOS (2 cases) subtypes. Immunohistochemical staining for desmin was positive in 10/10. For skeletal specific markers, MYOD1 was positive in 4/4 and myogenin in 7/8. The one myogenin negative case was a pleomorphic RMS that was positive for MYOD1. Three cases in the adult subset (2 NOS, 1 pleomorphic) demonstrated varying degrees of positive staining for cytokeratins, with one case strongly positive. Follow-up information was available on 3 cases. Two pediatric cases with follow-up demonstrated recurrence (embryonal at 20 months, alveolar at 14 months) and ultimately died of disease after 9 years and 3 years, respectively. A single adult patient with pleomorphic RMS was ANED after 1 year.

Conclusions: Primary cutaneous RMS demonstrates a distinctly bimodal age distribution that correlates with RMS in other settings. Cutaneous embryonal and alveolar RMS were more common in children, while cutaneous pleomorphic RMS is most common in adults. Immunoreactivity for cytokeratin was seen in 3 adult cases, a diagnostic pitfall in the differential diagnosis with sarcomatoid carcinoma. Recognition of rhabdomyoblastic cytologic features should prompt consideration of RMS. Based on limited follow-up, primary cutaneous RMS may still behave aggressively, especially in children.

520 A Ratio of miR-30b/miR-205 Is Accurate in Distinguishing Spitz Nevus from Primary Cutaneous Melanoma

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Background: Distinguishing Spitz nevus (SN) from Primary Melanoma (PM) remains a difficult histopathologic diagnosis. Recently, microRNA (miRNA) expression has been successfully used to classify tumors into appropriate lineages. We speculated that miRNA profiles differ in Spitz nevus as compared to primary melanoma, and thus could be used as a diagnostic aid.

Design: To address this, we compared the miRNA expression profiles of 14 classic Spitz nevi and 47 primary melanoma tumors using archival formalin fixed, paraffin embedded (FFPE) tissue. We have previously confirmed the validity of using FFPE tissue to profile miRNAs using an array platform. An Agilent miRNA microarray platform was used to generate miRNA expression profiles.

Results: We found that unsupervised hierarchical clustering clearly separated Spitz nevi from primary melanoma samples. Furthermore, Significance Analysis of Microarray (SAM) was applied to identify differentially expressed miRNA. We found both up-regulated and down-regulated miRNAs in primary melanoma relative to Spitz nevi. Two of the most significantly dysregulated miRNAs were miR-30b and miR-205. When used as a ratio, miR-30b/miR-205, we were able to accurately classify Spitz Nevi. The mean value for the miR-30b/miR-205 ratio in Primary Melanoma was 13.3 (range 0.036-105.7; n=47). The mean value for the miR-30b/miR-205 ratio in Spitz nevi was 0.004 (range 0.001-0.011; n=14). The p-value for the ratio of miR-30b/miR-205 between PM and SN found using an independent t-test is 0.003. To technically validate the microarray data, we conducted qRT-PCR on 15 PM and 6 SN samples from the original cohort profiled by microarray. The mean CT difference between miR-205 and miR-30b from qRT-PCR data for PM was 6.3 (standard deviation 4.1), and for SN was 0.39 (standard deviation 0.93). The p-value from an independent t-test using the qRT-PCR cohort was lower than 0.001. Our data showed a significant correlation between matched samples from microarray and qRT-PCR data (Kendall tau, 0.800; Spearman rho, 0.929).

Conclusions: In conclusion, we have found that the ratio of two miRNAs is highly accurate as a diagnostic test for Spitz Nevus.

521 Expression of ERG, an Ets Family Transcription Factor, Distinguishes Cutaneous Angiosarcoma from Histologic Mimics

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Background: Cutaneous angiosarcoma (AS) is most common on the scalp and face of elderly adults. The diagnosis of vasoformative AS is generally straightforward, whereas poorly differentiated AS can be difficult to distinguish from other neoplasms that arise in sun-damaged skin, such as squamous cell carcinoma (SCC), melanoma (MM), atypical fibroxanthoma (AFX), and leiomyosarcoma (LMS). A recent study showed that ERG, an Ets family transcription factor, is a sensitive and specific marker for endothelial differentiation. However, ERG expression has not been evaluated in non-AS cutaneous tumors. The purpose of this study was (1) to determine if immunohistochemistry (IHC) for ERG can distinguish cutaneous AS from histologic mimics and (2) to compare expression of ERG with FLI1, another Ets transcription factor widely expressed in vascular lesions.

Design: In total, 71 cutaneous head and neck tumors were retrieved from surgical files, including 22 AS (7 spindle cell, 4 epithelioid), 15 poorly differentiated SCC (5 spindle cell), 17 MM (8 spindle cell, 1 pseudovascular), 12 AFX (6 spindle cell, 6 pleomorphic), and 5 LMS. IHC was performed following antigen retrieval using a rabbit anti-ERG monoclonal antibody (1:2000; EPR3864(2); Epitomics) and a mouse anti-FLI1 monoclonal antibody (1:100; G146-222; BD Biosciences). The extent of immunoreactivity was graded according to the percentage of positive tumor cell nuclei (0, no staining; 1+, <5%; 2+, 5% to 25%; 3+, 26% to 50%; 4+, 51% to 75%; 5+, 76% to 100%), and the intensity was graded as weak, moderate, or strong.

Results: Distinct nuclear staining for ERG was observed in all 22 AS cases (95% strong 5+). All other tumor types were negative for ERG. FLI1 showed strong, diffuse nuclear staining in all AS cases. However, FLI1 was also positive in 13 of 15 (87%) SCC (40% moderate or strong; all 4-5+), 10 of 17 (59%) MM (12% moderate; variable extent), 11 of 12 (92%) AFX (25% moderate; variable extent), and 1 of 5 (20%) LMS (strong 5+). The sensitivity and specificity of ERG for AS are both 100%. The sensitivity of FLI1 for AS is also 100%, but the specificity is only 29%; if only moderate-strong staining is considered positive, the specificity is 76%.
Conclusions: ERG is a highly sensitive and specific marker for cutaneous AS and typically shows strong, diffuse nuclear staining. FLI1 is also highly sensitive for AS, but shows limited specificity. IHC for ERG is useful to support the diagnosis of AS, particularly in poorly differentiated cases.

522 Pleomorphic Dermal Sarcoma: Clinicopathologic Analysis of 32 Cases

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Background: If strict morphological and immunophenotypic criteria are applied, the behavior of atypical fibroxanthoma is almost entirely benign. However, tumors with similar pathological features but deep subcutaneous invasion, necrosis, lymphovascular or perineurial invasion may be associated with adverse outcome. Such tumors may be best regarded as pleomorphic dermal sarcoma (PDS). As this concept is not universally accepted, we aim to further characterize their clinical and morphological features as well as behavior.
Design: 32 PDS were retrieved from the departmental files at NHS Lothian, Edinburgh, UK. Hematoxylin and eosin-stained tissue sections as well as immunohistochemistry for multiple markers were assessed to exclude epithelial, melanocytic, smooth muscle and endothelial differentiation. Clinical and follow-up data were obtained.
Results: Median age at presentation was 81 years (range: 47-96) with a strong male predilection. All tumors occurred on sun-damaged skin, mostly of the scalp. Only one tumor was located outside the head and neck area.
Tumors were nodular or polypoid with median thickness of 11.5mm (range: 3.5-26). When available for assessment, all tumors invaded at least into deep subcutis. Skeletal muscle or fascia involvement was identified in 82%, and ulceration in 78% of cases. The tumors were composed of fascicles of atypical spindle cells, or less frequently of sheets of pleomorphic epithelioid cells. A subset of tumors showed mixed features and multinucleated tumor giant cells were admixed in varying proportions. Additional findings were myxoid, desmoplastic, pseudoangiomatous, keloidal or storiform growth patterns. The mitotic count was brisk, including atypical forms, and tumor necrosis was observed in 22%. Lymphovascular invasion was noted in 5, perineurial infiltration in 7 patients.
Multiple cytokeratin markers, S100, HMB-45, desmin and CD34 were negative. SMA was expressed in 70% of cases, EMA in 16%, CD31 in 48%, Melan A in 6% and p63 in 1 case only.
Follow-up (median, 20 months) was available for 28 patients. 6 patients recurred locally with no further recurrences in 4 patients. 1 patient developed further recurrences as well as multiple cutaneous metastases, and died after 13 months. 5 patients died from unrelated and 5 from unknown cases.
Conclusions: This study outlines the clinical and morphological features of PDS. Tumors may recur locally but distant metastasis and disease related death is rare despite the deep invasion and presence of lymphovascular invasion.

523 Adoption of FISH for Diagnosis of Melanoma

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Background: The distinction between benign nevi and malignant melanoma (MM) can at times be difficult or impossible based on morphology and immunohistochemical analysis. Fluorescence in situ hybridization (FISH) is a molecular tool which has become diagnostically important in dermatopathology. Gerami et al (Am J Surg Pathol 2009 Aug;33(8):1146-56) were the first to demonstrate a four color FISH probe assay (RREB1, CCND1, CEN6 and MYB) which showed high sensitivity and specificity for distinguishing benign nevi from MM. The purpose of this study was to establish this FISH assay at our laboratory and confirm the utility of the assay using archival nevi and MM.
Design: The RREB1 probe was synthesized in-house via nick translation using a BAC clone (RP11-61016) labeled with SpectrumRed (Abbott Molecular Inc, IL). The other probes are commercially available. Specimens were selected on the basis of morphological diagnosis. Ten MM and 5 nevi were interrogated with FISH. The specimens were analyzed for number of signals corresponding to each probe and interpreted using published criteria (see Table 1). If the specimen fulfilled any of the criteria, it was considered a positive result.
Evaluation of Spitz nevi and atypical spitzoid tumors with this 4-probe set is ongoing. Currently, optimization of two additional FISH probes targeting 9p21 (*p16*) and 11p15 (*HRAS*) is underway. Benign nevi, MM, Spitz nevi and atypical spitzoid tumors will be interrogated with these additional probes.
Results: One of the MM cases failed due to tissue processing error. The remaining 9 MM cases showed positivity for one or more criteria for melanoma. Of the 5 nevi cases, none showed positivity for any of the criteria.

Table 1: Criteria of FISH assay for MM

Morphologic Diagnosis	>29% cells have >2 RREB1	>55% cells have RREB1>CEN6	>40% cells have MYB<CEN6	>38% cells have > 2 CCND1
nevus, intradermal	N 3%	N 31%	N 9%	N 0%
nevus, intradermal	N 2%	N 8%	N 16%	N 8%
nevus, compound	N 0%	N 16%	N 14%	N 4%
nevus, compound	N 2%	N 8%	N 16%	N 14%
nevus, compound	N 8%	N 14%	N 6%	N 8%
MM, nodular	Y 82%	Y 90%	N 6%	N 12%
MM, nodular	Y 60%	Y 64%	Y 40%	N 16%
MM, nodular	Y 92%	Y 100%	Y 50%	Y 40%
MM, superficial spreading	Y 50%	Y 46%	Y 40%	N 12%
MM, spindle	Y 53%	N 40%	N 16%	N 5%
MM, metastatic	Y 80%	Y 80%	N 18%	N 20%
MM, metastatic	Y 54%	N 50%	N 20%	Y 84%
MM, metastatic	Y 70%	Y 58%	Y 62%	N 5%
MM, metastatic	Y 72%	Y 84%	N 18%	N 2%

Conclusions: Our study confirms the utility of the four probe FISH assay for distinguishing benign nevi from MM using archival material. This technique has the potential to aid in diagnosis of MM in morphologically difficult cases.

524 Evaluation of Single Cell Metastasis in Melanoma; Two New False Staining Patterns

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Background: The American Joint Commission on Cancer (AJCC) 2010 guideline changes recommend all patients with single cell metastasis identified by immunhistochemical (IHC) staining to be classified as stage III disease. The recommendation further says that one positive cell by IHC staining with an "atypical appearance" warrants upstaging. Advancement to stage III, especially for young patients, often results in adjuvant therapies and inclusion in clinical trials with significant side effects. These guidelines place enormous confidence on IHC techniques and responsibility on the pathologist to discern whether a single chromogen covered cell truly represents a malignant melanocyte. This study aims to identify the frequency and pattern of false staining by MITF, Melan A, and HMB-45.
Design: 20 benign lymph nodes from non-melanoma patients were stained using MITF (Ventana, Tucson, AZ), Melan A (Ventana, Tucson, AZ), and HMB-45 (Leica, Newcastle, UK) with appropriate negative controls. H&E and IHC stained sections were assessed for the frequency of positive staining, pattern and number of cells stained, location of positive cells, present of pigment and perceived atypia.
Results: 17/20 (85%) of cases evaluated showed >1 cell staining with at least one IHC. 16/17 (94.1%) of the positive cases had cells located subcapsular. 14/17 (82.4%) cases showed staining on at least 2 different stains. MITF nuclear stained cells were seen in all 17 cases whereas Melan A was only seen in 6/17 cases (35.3%). HMB-45 was positive in 10/17 (58.5%) cases and correlated with Melan A staining 85% of the time (17/20). Two distinct staining patterns were noted in the MITF positive stained cases. The first pattern showed multiple nuclear and cytoplasmic positive clusters of large, atypical appearing cells in 11/17 (67.7%) cases with 9/11 (81.8%) of these having visible nodal pigment on H&E. A second pattern showed multiple, single, small, typical appearing cells with nuclear only positivity in 6/17 (35.3%) cases with only 2/6 (33.3%) of these having visible nodal pigment on H&E.
Conclusions: The new guidelines for positivity in sentinel nodes has placed much weight on the use of IHC to identify single cell metastasis in melanoma patients. False positive staining using nuclear MITF is even higher than the previous reports for Melan A and appears increased in pigmented nodes. Upstaging of melanoma patients is thus solely placed on the pathologist's ability to discern patterns of false staining as described here and the subjective identification of atypia in single stained cells.

525 Interobserver Agreement of Assessment of Desmoplasia and Neurotropism in Melanoma

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Background: A proposed scheme for classification of desmoplastic melanoma (DM) divides melanomas that exhibit desmoplasia into pure DM [pDM, in which ≥90% of invasive tumor is desmoplastic] and combined DM [cDM, in which <90% of invasive tumor is desmoplastic]. This classification of DM type correlated with survival in some studies but not in others. One possible reason for these conflicting findings is that the assessment and classification of desmoplasia is not reproducible. We sought to determine the level of interobserver agreement in the classification of desmoplasia and neurotropism in melanoma.
Design: Hematoxylin-eosin-stained slides from 50 cases of melanoma exhibiting varying degrees of desmoplasia and neurotropism were scored by 6 pathologists. Each observer scored the following: DM type (pDM or cDM); nerve involvement (absent, focal or extensive); location of nerve involvement (within or outside main tumor mass, or both); and whether the tumor should be termed "neurotropic melanoma". The level of interobserver agreement for each parameter was assessed using kappa (k) statistic.
Results: Agreement for assessment of DM type, nerve involvement, location of nerve involvement, and whether the observer would classify the tumor as "neurotropic melanoma" were moderate (k=0.47), fair (k=0.31), slight (k=0.13) and fair (0.39), respectively (Table).

Observer->	1*	2*	3*	4*	5*	6*	Kappa
DM type							0.47
Pure DM	22(44)	37(74)	30(60)	33(66)	32(64)	23(46)	
Combined DM	18(36)	13(26)	12(24)	13(26)	15(30)	19(38)	
Not DM	10(20)	0	8(16)	4(8)	3(6)	8(16)	
Nerve involvement							0.31
Absent	17(34)	26(52)	30(60)	24(48)	10(20)	29(58)	
Focal	9(18)	23(46)	3(6)	5(10)	6(12)	2(4)	
Extensive	24(48)	1(2)	17(34)	21(42)	34(68)	19(38)	
Location of nerve involvement							0.13
Within main tumor mass	11(33)	3(13)	12(60)	6(23)	15(37)	5(24)	
External to main tumor mass	3(9)	18(75)	1(5)	8(31)	7(18)	2(10)	
Both within and external	19(58)	3(13)	7(35)	12(46)	18(45)	14(67)	
Neurotropic melanoma							0.39
No	30(60)	27(54)	32(64)	24(48)	10(20)	29(58)	
Yes	20(40)	23(46)	18(36)	26(52)	40(80)	21(42)	

*no. of cases (%)

Conclusions: There was only moderate agreement in the assignment of DM type, which may explain the disparate findings of studies assessing the prognostic significance of this classification system. Agreement in the assessment of neurotropism was poorer. Clearer definitions and criteria are required to improve the reproducibility of assessment of desmoplasia and neurotropism in melanoma, and to evaluate their true prognostic value.

526 Lymphovascular Markers in Melanoma Sentinel Lymph Nodes

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Background: Cutaneous malignant melanomas principally metastasize to lymph nodes, and sentinel lymph node (SLN) sampling has become a critical facet of melanoma prognosis and treatment. Evidence has shown that melanomas trigger lymphangiogenesis at the tumor-stromal junction and that this process is correlated with SLN metastasis. Further, there is evidence that melanomas may produce premetastatic niches in SLNs through a similar process of influencing lymphangiogenesis. SLNs excised in the treatment of melanomas were retrospectively stained for lymphovascular immunohistochemical markers (CD31, CD34, D2-40) to identify changes in intranodal vascular density and correlate those changes with clinical outcomes.

Design: 65 melanomas with SLN biopsy, clinical follow-up, and adequate paraffin-embedded nodal tissue were studied. Immunohistochemistry for CD31, CD34, and D2-40 was performed. Stained slides were analyzed for microvessel density (MVD) based upon the manual quantitation of vessels within three 20x high-powered fields over "hot spots". Automated quantitation of staining density and intensity was performed using a Dako ACIS III scanner. Mean MVDs, mean staining percentages (% stain), and mean staining intensities were compared.

Results: 65 melanoma patients included 8 with negative SLNs and recurrence, 26 with negative SLNs and no recurrence, and 31 positive nodes with and without recurrence.

		CD31			D2-40		
	Number	Mean MVD	% Stain	Intensity	Mean MVD	% Stain	Intensity
Negative with recurrence	8	78.5*	50.1	101.4*	9.1	22.5*	69.4*
Negative without recurrence	26	117.5*	44.2	84.2*	6.2	13.1*	71.3*
Positive	31	93.7*	37.4	89.4*	7.2	7.1*	63.8*

(* indicates $p < 0.05$)

D2-40 % staining by image cytometry is significantly greater in the negative SLNs with recurrence than in the negative SLNs without recurrence ($p = 0.002$) and in the positive SLNs ($p = 0.001$). D2-40 and CD31 intensity is significantly greater in negative SLNs with recurrence than in positive SLNs ($p = 0.003$, $p = 0.04$, respectively); CD31 intensity is significantly greater in negative SLNs with recurrence than in those without recurrence ($p = 0.001$). CD34, both visually and by image cytometry, showed no significant differences amongst the three groups.

Conclusions: D2-40 and CD31 image cytometry shows more lymphatic and vascular channels in negative SLNs with subsequent recurrence than in those without recurrence and in positive SLNs. This indicates increased lymphangiogenesis and angiogenesis in SLNs prior to melanoma metastasis.

527 Prognostic Significance of CD30 and PDL1 Expression in Patients with MF in Early-Stage Disease

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Background: Mycosis fungoides (MF) is a non-Hodgkin's T-cell lymphoma of the skin that often begins as limited patches and plaques with slow progression to systemic involvement. We objective was evaluated the prognostic value of CD30 and PDL1 in patients with MF in early-stage disease.

Design: We have analyzed 33 mycosis fungoides (MF) presented with early-stage disease IA-IB. All the cases were retrospectively evaluated analysing their histological and clinical data. Immunohistochemistry was performed in tissues fixed in buffered formalin and embedded in paraffin. The following antibodies were used : CD20, CD3, CD4, CD8, CD30, PDL1.

Results: Disease progression (lymph node y/o bone marrow) occurred only in 18,2%, of cases and 6,3% of patients died due to MF/SS. The mean age at diagnosis was 60,18 years. There were 45% of cases with recurrence after of treatment (corticosteroid and phototherapy) and 69,7% achieved a complete clinical response, with a following up of 34,16 months of mean. We found small-medium –sized lymphocytes CD30 positive (<25%) in 5 of 33 cases. Twenty-nine (87,9%) from 33 cases showed immunoreactivity for PD1L and there were 4 cases with loss of expression for PDL1. We observed that 50% (3) of the cases that showed systemic involvement were negative for PDL1. While that only a 4,34% of the cases positives for PDL1 showed systemic progression (p:0,018). We also observed a relation between the presence of cells CD30 and the systemic involvement (p:0,04). There was no statistically significative relation between the loss of reactivity for PD1L with the recurrence or completed clinical response.

Conclusions: The loss of expression for PDL1 and the presence of CD30 positive cells could be considered parameters associated with poor prognosis in MF in early-stage.

528 MITF Is the Most Effective Melanocytic Marker for Evaluation of Atypical Intraepidermal Melanocytic Proliferations

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Background: Atypical intraepidermal melanocytic proliferations (AIMP) are lesions along the spectrum between actinic lentigo and melanoma in situ. They are non-nested, pagetoid and/or lentiginous melanocytic proliferations for which a definitive diagnosis is difficult to provide. Immunohistochemistry for S-100, MITF and Melan A is commonly used to aid differentiation of these entities. However, we have noticed that Melan-A, MITF and S-100 all appear to stain different numbers of cells in the epidermis. We attempt to determine the best IHC stains for evaluation of epidermal melanocytes.

Design: 49 specimens with a diagnosis of AIMP were selected from historical archives. Appropriate formalin fixed paraffin embedded blocks containing representative lesional tissue were cut and immunohistochemically stained with S-100, Melan A and MITF. A representative area of AIMP was selected on H&E and the fraction of cells at the dermal-epidermal junction that are melanocytes was evaluated. The results were independently determined by three pathologists.

Results: S-100 and Melan-A demonstrate cytoplasmic staining and MITF demonstrates nuclear staining. Melan-A stains a significantly higher percentage of cells at the dermal epidermal junction (mean 55% of cells were positive) than any other stain. MITF stains 34% of the cells near the dermal epidermal junction, which is similar to the percentage of melanocytes identified by H&E. S-100 stains the fewest number of cells (mean 9%). The difference between MITF and H&E is not statistically significant. Melan-A stains the highest percentage of cells in 47 of the 49 specimens evaluated.

Conclusions: Different immunohistochemical stains are liable to produce distinct estimates of the number and confluence of melanocytes at the dermal-epidermal junction. Due to the cytoplasmic staining pattern of Melan A, junctional melanocytes may often be overinterpreted. S100 staining, although cytoplasmic, is often weak and patchy. MITF produces results that most closely approximate those of H&E. Therefore, MITF is an effective stain for detecting melanocytes in AIMP, and should be preferentially used for evaluation of these lesions.

529 Comparison between the Conventional and Phospho-Histone H3 (PHH3) Immunohistochemistry-Assisted Mitotic Count in Different Subtypes of Melanomas

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Background: The mitotic count in melanocytic lesions is an important diagnostic and prognostic factor. It can be difficult to reliably identify mitotic figures in H&E stained sections. We investigated whether mitotic count using Phospho-Histone H3 (PHH3) immunohistochemistry would produce less inter-observer variability in comparison with H&E stained sections. Another aim of the study was to see whether PHH3 would be helpful in the diagnosis of nevoid and desmoplastic melanomas.

Design: Formalin-fixed, paraffin-embedded tissue of 110 conventional invasive, 9 nevoid and 8 desmoplastic melanomas were used in this study. Sections from these 127 tumours were stained with H&E and PHH3. Mitotic count per 1 mm^2 was performed in both H&E and PHH3-stained sections independently by two pathologists and by two histotechnologists. Intradermal nevi were used as a negative control on each slide. Data were assessed by the Bland-Altman (BA) analysis.

Results: Combined mean mitotic count for all cases on PHH3 stained sections was three times higher than on H&E for all of the participants as a group. Comparison of the mitotic count on H&E between pathologists and histotechnologists showed poor reproducibility with BA analysis showing mean difference -7.25 [CI -28.25-14.37]). In contrast, comparison of mitotic counts between pathologists and histotechnologists on PHH3 stain resulted in BA mean difference -0.34 [CI -2.75-2.11]), indicating good reproducibility between the participants. Mitoses were easily identified in PHH3 stain in nevoid and desmoplastic melanomas. Of 127 nevi only 18 showed 1-2 mitoses per slide in PHH3 stained sections.

Conclusions: PHH3 facilitates the mitotic count. Number of mitoses in melanomas assessed by PHH-3 staining is three times higher than by H&E. PHH3 allows for more reproducible mitotic counts than by H&E. PHH3 confirms mitotic inertness of the intradermal nevi, while clearly highlighting deep dermal mitoses in nevoid and desmoplastic melanomas.

530 Tandem Mass Spectrometry Study Using Micro-Dissected Epithelial Cells from Psoriasis and Chronic Eczematous Dermatitis

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Background: Psoriasis and chronic eczematous dermatitis are clinically distinct entities. However there is an overlap in their histopathologic appearance. Additionally, the molecular mechanisms leading to each disease state are quite different. Previous studies in our lab have validated the use of tandem mass spectroscopy in preparations made from formalin fixed paraffin embedded tissue (FFPE) to identify altered protein expression in cutaneous tumors. This study aims to determine if this method can be used to identify differentially expressed proteins in inflammatory lesions such as psoriasis and chronic eczematous dermatitis. Additionally, proteins that are variably expressed will then be the target for further analysis.

Design: Epidermal cells from 4 cases of psoriasis or chronic eczematous dermatitis were harvested via laser micro-dissection. The proteins were extracted and analyzed

by Thermo-LTQ-XL mass spectrometer coupled to an Eksigent nanoLC-2D. Using NSAF spectral counting techniques, we were able to normalize and compare protein levels between samples. Anything with 1.5 fold difference is considered to be a significant difference.
Results: 891 distinct proteins were identified in these samples. Interestingly, only 15 proteins were found to be up-regulated in psoriasis and 3 proteins were found to be down-regulated in psoriasis. Only three of these proteins have been published in the literature as having misregulated expression in psoriasis. From this list, we have chose cytokeratin 10 and CCT3 (chaperon containing TCP3) proteins, to validate the mass spectrometry results using immunohistochemical stains. Both of these proteins appear to be increased in psoriatic lesions when compared to normal epidermis and eczematous lesions.
Conclusions: This study demonstrates that laser micro-dissected FFPE sections coupled with mass spectrometry can even identify biomarkers for inflammatory lesions. Finding only 18 proteins that have significant expression differences between psoriasis and eczema is not surprising, because histologically these two lesions have many overlapping features. Our findings can be validated by immunohistochemistry, which may then be used to distinguish psoriasis and chronic eczematous dermatitis.

531 SOX10 Expression Distinguishes Desmoplastic Melanoma from Its Histologic Mimics

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Background: Desmoplastic melanoma (DM) presents diagnostic challenges due to histologic mimics such as spindle cell squamous cell carcinoma, atypical fibroxanthoma (AFX) and sarcoma. While S100 usually stains DM, other melanoma markers (HMB-45, Melan-A and tyrosinase) are often negative. DM's mimics often show negative or unreliable immunohistochemical staining. SOX10 is a transcription factor that plays a role in schwannian and melanocytic cell development. The nuclear staining pattern of SOX10 transcription factor provides a clean, reliable and interpretable stain. Recently, SOX10 expression has been shown to be a very sensitive and specific marker of desmoplastic melanoma. Currently, there are no large studies comparing the sensitivity and specificity of SOX10 for desmoplastic melanoma among its most common histologic mimics.
Design: 77 cases were retrieved from the UCLA Pathology archives, including : DM (N=16), spindle cell/poorly-differentiated carcinoma (SC – N= 18), AFX (N=13), sarcoma with spindled morphology (N=20), and malignant peripheral nerve sheath tumor (MPNST – N=10). 75% (15/20) of sarcoma cases were centered in the dermis or subcutaneous tissues and included DFSP with sarcomatous transformation, myofibroblastic sarcoma, synovial sarcoma and sarcoma not otherwise specified. H&E slides were reviewed and SOX10 immunohistochemistry was performed on all cases.
Results: SOX10 was strongly, diffusely positive in 100% (16/16) of DMs and showed focal staining in 30% (3/10) of MPNSTs. All other tumors were negative for SOX10 (0% (0/18) of SCs, 0% (0/13) of AFXs, 0% (0/20) of sarcomas). SOX10 showed nuclear positivity only, as previously reported.
Conclusions: SOX10 is a sensitive marker for desmoplastic melanoma, demonstrating 100% sensitivity in this study. Importantly, SOX10 was negative in all histologic mimics that commonly occur in the dermis, including SC, AFX and sarcomas. Similar to S-100 protein, some MPNSTs show scattered positivity, but do not show diffuse positivity as in the desmoplastic melanomas. In conclusion, SOX10 is a useful marker to confirm the diagnosis of DM and to exclude its histologic mimics.

532 Cutaneous CD30 + Lymphoproliferative Disorders with CD8 Expression: A Clinicopathological Study of 18 Cases

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Background: Lymphomatoid papulosis (LyP) and primary cutaneous anaplastic large cell lymphoma (PALCL) belong to the group of CD30+ lymphoproliferative disorders. LyP is a self-healing disorder with "waxing and waning" papules and nodules. The disease is characterized by a favorable prognosis; however, secondary lymphomas like mycosis fungoides or Hodgkin disease may develop. PCALCL represents one end of the spectrum of CD30 (+) lymphoproliferative disorders and it has been generally regarded as clinically indolent lymphoma. There is limited precedent literature regarding cytotoxic profile in cutaneous CD30+ lymphoproliferative disorders and there are only rare cases reported in the literature. The purpose of this study is to highlighty the clinical, light microscopic,and phenotypic features of CD8+ cutaneous CD30 lymphoproliferative disorders emphasizing the differential diagnostic distinction from more agrresive T-cell lymphomas.
Design: The diagnoses of CD30+ lymphoproliferative disorders were based on standard published histopathologic criteria and results of immunohistochemical studies at the time of initial diagnosis. We describe 18 cases (14 males, 17-77 years old; 4 females, 10-61 years old) of CD30+ lymphoproliferative lesions expressing CD8 / CD30 immunophenotype. 14 cases were compatible with PCALCL and 4 cases with LyP.
Results: All of our cases showed co-expression of CD30 and CD8. Follow-up data available in all patients revealed that two cases had associated systemic disease involvement and two cases had associated mycosis fungoides and chronic lymphocytic leukemia. Histologically, one case of ALCL showed prominent myxoid changes, one case of ALCL had extensive intravascular involvement, and one case of LyP was clasified as type D.
Conclusions: There is limited precedent literature regarding cutaneous CD30+ lymphoproliferative disorders with CD8 coexpression. Most cytotoxic T-cell lymphomas demonstrate an aggressive clinical course and there is a low threshold to initiate cytotoxic therapy in this setting. In cases of cutaneous CD8/CD30 co-expression this should prompt a careful clinical history for associated systemic disease and previous self-resolving lesions. Also, this cytotoxic profile may create diagnostic pitfalls as may be histopathologically indistinguishable from more aggressive lymphomas such as primary cutaneous aggressive epidermotropic CD8+ cytotoxic T-cell lymphoma.

533 Mismatch Repair Protein Immunohistochemistry in Sebaceous Lesions

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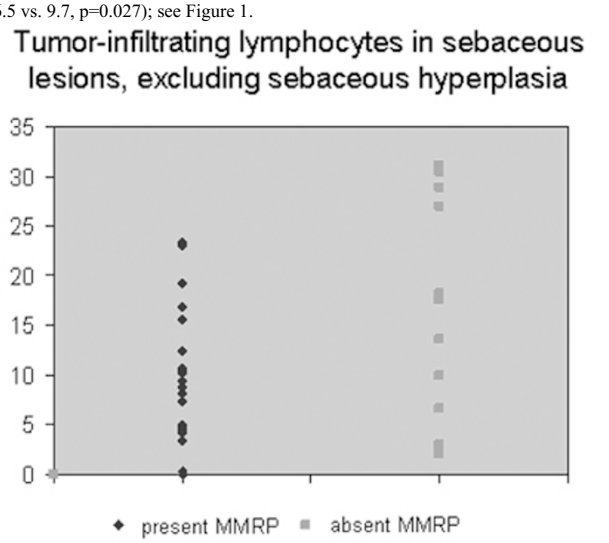
Background: The association between Lynch syndrome and sebaceous neoplasms is well-characterized. Absence of expression of mismatch repair proteins (MMRP) by immunohistochemistry is often used in other Lynch-associated tumors such as colon and endometrial carcinomas to guide testing for this disorder.
Design: Immunohistochemical staining for the MMRP MLH1, PMS2, MSH2, and MSH6 was performed on 36 sebaceous neoplasms and 7 cases of sebaceous hyperplasia and presence or absence of staining was assessed. Additionally, number of tumor-infiltrating lymphocytes per high-powered field (TIL) was counted using the H&E stained slide, averaged over 5-10 high-powered fields. Medical records were also reviewed.
Results: The composition of the 43 sebaceous lesions examined and their immunohistochemical profiles are summarized in Table 1.

MMRP expression in sebaceous lesions.

lesion	MMRP present	≥1 MMRP absent	total
sebaceous carcinoma	11	4	15
sebaceous neoplasm	3	9	12
sebaceous adenoma	5	1	6
sebaceoma	3	0	3
sebaceous hyperplasia	7	0	7
	29	14	43

Of the neoplastic sebaceous lesions, 38.9% lacked expression of one or more MMRP. In 10 of the 14 lesions with absent MMRP expression, both MSH2 and MSH6 were absent. Three lacked MLH1 and PMS2 expression and one lesion lacked only MSH6. Of the 10 patients with absent MMRP expression, 2 had previously diagnosed Lynch syndrome, 3 had some combination of personal and/or family history of endometrial and/or colon carcinomas, 3 had no personal or family history suggestive of Lynch syndrome, and 2 had no recorded personal or family history. A total of two patients had multiple sebaceous lesions and both patients lacked expression of MSH2 and MSH6 in all lesions. One of those patients had a diagnosis of Lynch syndrome and the other had a family history of uterine cancer. TIL in patients with absent MMRP expression was statistically significantly greater than in patients with normal MMRP expression (16.5 vs. 9.7, p=0.027); see Figure 1.



Conclusions: Sebaceous lesions other than hyperplasia are rare entities that frequently lack MMRP expression. These neoplasms are commonly associated with Lynch syndrome and immunohistochemical screening for MMRP expression might help select for patients in whom genetic testing is indicated.

534 C-Kit Protein Expression in Female Lower Genital Tract Melanoma

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Background: Vulvar and vaginal melanomas represent 37.1% and 7.4% of the mucosal melanomas respectively. C-Kit is a tyrosine kinase receptor altered in up to 40% of mucosal melanomas. Tyrosine kinase inhibitors (TKI) are effective in mucosal melanomas harboring specific KIT gene mutations. An association between mutated KIT and expression of C-Kit by immunohistochemistry (IHC) has been suggested. Our study explores C-Kit IHC expression in vulvovaginal melanomas and its correlation with prognostic factors.
Design: Formalin-fixed paraffin-embedded tissue blocks of vulvovaginal melanomas from 23 patients diagnosed between 1987 and 2009 were retrieved from our files. Demographic, anatomic location and prognostic data were recorded. Protein expression was semi-quantitavely scored in sections immunostained with C-Kit (DAKO 1:1000) assessing staining percentage (<20%:1+,20-50%:2+,>50%:3+) and intensity (0 to 3+)

with a cumulative score ranging from 0 to 6 (0-2 negative, 3 borderline, 4-6 positive). The data was subjected to multiple regression analysis considering known melanoma prognostic factors.

Results: The age ranged from 44 to 90 years old (mean 70.8 n=23). Sixteen tumors were vulvar (70%) with 14 labial and 2 clitoral. Seven tumors (30%) were vaginal. The tumors were bulky (mean thickness of 6.12 mm.) with 17 (72%) level IV lesions (72%). Ulceration was present in 15 (65%) tumors and mitotic activity was brisk (mean mitotic count 5.4 per mm^2). Lymphovascular invasion (LVI) was found in 5 (22%) cases. Scoring of IHC slides yielded 3 negative, 5 borderline and 15 positive cases (mean cumulative score 4.3). Positive/borderline cases (97% n=20) were composed of four cases staining only the melanoma in-situ component and 17 cases staining the invasive component (only in RPG:2 cases, VGP:14 cases). Multiple regression analysis revealed that C-Kit expression was significantly lower in level IV lesions compared to level II-III lesions (p=0.014) after adjusting for other significant factors. Presence of LVI further decreased C-Kit score by 2.3 (p=0.001). This data confirms that of previous studies in melanomas from other anatomical sites regarding relative loss of C-Kit expression with progression of disease.

Conclusions: We found that a great proportion (97%) of our 23 cases of female genital tract melanomas expressed C-Kit protein by IHC and that it was comparatively diminished with disease progression. In our opinion, although tempting, the association between C-Kit expression and TKI-sensitive KIT mutations in melanomas should be confirmed with genetic analysis.

535 BRAF V600E and V600K in Melanoma: Clinicopathologic Correlation

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Background: The aim of this study was to compare the clinicopathologic correlates of two most common BRAF mutations in melanomas: V600E and V600K.

Design: Patients with melanomas harboring BRAF V600E or V600K mutations were identified among cases consecutively tested for BRAF mutations as part of routine clinical management. Sanger sequencing was performed on formalin-fixed paraffin embedded samples. The type of BRAF mutation, disease-free survival (DFS), and clinicopathologic features were tabulated and analyzed. To characterize BRAF mutant allele specific imbalance (MASI), peak heights of BRAF mutant allele (MA) and wild type allele (WTA) as seen on forward sequencing electropherograms (SE) were compared and categorized in 2 groups: MA>WTA, i.e., MASI, or MA≤WTA. Eight cases of MA>WTA and 3 cases of MA≤WTA were selected for BRAF fluorescence in situ hybridization (FISH) with BRAF/CEP7 dual color probe (Abbott Molecular, Inc., Des Plaines, IL). BRAF /CEP7 ratio ≥ 2 was considered positive for amplification. Polysomy was defined as > 2 chromosome enumeration probe 7 signals in ≥ 40% of cells.

Results: Patients with BRAF V600E (n=64) were on average 17 years younger than patients with BRAF V600K melanomas (n = 18) (47 years versus 64 years of age, p<0.001). MASI was more commonly seen in V600E than in V600K mutations (44/64, 69%, versus 7/18, 39%, p < 0.001). Cases with BRAF MA>WTA on SE showed BRAF amplification (3/8 cases), chromosome 7 polysomy (5/7 cases) and chromosome 7 monosomy (1/8 cases) on FISH. Patients with BRAF V600K melanomas had shorter DFS compared to BRAF V600E melanomas (mean DFS 18.4 months, 95% confidence interval [95 CI] 8.8 – 28 versus 64.8, 95 CI 44 – 85 months, respectively, p < 0.001). Age, gender, BRAF MASI and clinical stage did not correlate with DFS or overall survival.

Conclusions: BRAF V600K was found in 22% of cases in this cohort. BRAF MASI is more common in V600E than in V600K and develops by BRAF amplification, chromosome 7 polysomy and monosomy. Patients with BRAF V600K melanomas present at an older age and have a shorter DFS compared to BRAF V600E melanomas.

536 SOX10 Expression in Malignant Melanoma, Carcinomas, and Normal Tissues

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Background: SOX10 (Sry-related HMg-Box gene 10) is a nuclear transcription factor that plays an important role in melanocytic cell differentiation. It has been shown to be a sensitive marker of melanoma including spindle and desmoplastic subtypes. We assessed its expression in melanomas, carcinomas and benign tissues.

Design: In order to assess sensitivity and specificity, we studied melanomas, carcinomas, benign nevi, and normal tissues in tissue microarrays with routine immunohistochemistry for SOX10.

Results: All melanomas were strongly and diffusely nuclear SOX10-positive.They included 2 spindle cell, 1 desmoplastic and 2 in situ melanomas.

Type of tissue	Number of Cases	Sox10 Positive number/Percent
Primary melanoma	82	82(100%)
Metastatic melanoma	14	14(100%)
Ovarian cancer	24	0(0%)
Breast cancer	26	2(8%)
Endometrial cancer	23	0(0%)
Lung cancer	26	0(0%)
Colon cancer	25	0(0%)

Normal tissues	Number of Cases	Sox10 Positive number/Percent
Benign nevi	4	4(100%)
Ovary	2	0(0%)
Breast	8	8(100%)
Endometrium	4	0(0%)
Lung	5	0(0%)
Large bowel	5	0(0%)

Two of 26 (8%) breast carcinoma and all 4 benign nevi were SOX10 positive. The sensitivity and negative predictive value for SOX10 in the diagnosis of melanoma is 1.0 ; the specificity and positive predictive value is 0.98.

Conclusions: SOX10 is a sensitive, specific marker for melanoma. As benign nevi also express SOX10, it cannot be used as a differentiation marker between benign and malignant pigmented skin lesions. Only a small number of breast carcinoma (<10%) express SOX10; no carcinoma of ovary, endometrium, lung or colon expressed SOX10.

537 Nevus Density May Affect Melanoma Survival

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Background: Individuals with large numbers of nevi (>100) are at increased risk of developing melanoma, even in the absence of atypical nevi. Despite this knowledge, the association between number of nevi and survival with melanoma remains to be investigated.

Design: In our population based study of number of nevi and survival with melanoma, eligibility criteria included a diagnosis of non-metastatic primary invasive cutaneous melanoma. A questionnaire assessed patient characteristics, while a single dermatopathologist recorded histologic features of melanoma. Nevi were counted on the arms and backs of participants. Statistical analysis included simple frequencies, contingency tables and cross-tabulations, univariate models, and finally multivariate models. Covariates with statistically significant relationships to number of nevi or death were included in a multivariate analysis with death as the primary outcome.

Results: Our study assesses the survival of melanoma patients with a large number of nevi (approximately top 20th percentile), which corresponds to >30 nevi on the arms and back. Of all recorded variables only skin type, anatomic site, and skin spouse or self examination were found to have a statistically significant (p<0.05) association with number of nevi. None of these variables showed a significant association with death from melanoma. A multivariate analysis assessing survival was performed with pertinent patient and tumor variables. In the multivariate model, six factors showed a statistically significant relationship with survival (p<0.05), of which one was >30 nevi.

Independent predictors of death from melanoma in a multivariate model

Variable	Hazard ratio (95% confidernce interval)	P value
Number of nevi		
<30	1.0 (referent)	
30+	2.0 (1.2-3.3)	.01
Sex (forced into model)		
Female	1.0 (referent)	
Male	1.2 (0.8-2.0)	.35
Age at diagnosis (1 year increase)	1.0 (1.0-1.0)	.03
Solar elastosis		
Absent	1.0 (referent)	
Present	.58 (0.4-0.9)	.02
Melanoma site		
Other	1.0 (referent)	
Head/Neck	2.1 (1.2-3.7)	.01
Breslow thickness		
<0.76mm	1.0 (referent)	
0.76-1.69mm	2.1 (0.9-4.8)	.10
1.70-3.60mm	4.9 (2.1-11.6)	<.001
>3.60mm	6 (2.4-15.1)	<.001
Mitoses		
None	1.0 (referent)	
Any	3.5 (1.5-8.2)	.004

Conclusions: Individuals with melanoma and large numbers of nevi may exhibit decreased survival. This finding appears to be independent of the significant association of number of nevi with skin type, anatomic site of melanoma, and skin examination. Hypotheses to explain this relationship suggest that melanoma may progress through variably aggressive molecular pathways that are reflected by nevus density.

538 Pathology of Sentinel Lymph Nodes for Merkel Cell Carcinoma

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Background: In the present work, we evaluated the possibility of introducing ultrasonographic (US) examination in association to fine needle aspiration cytology (FNAC) before sentinel lymph node biopsy (SLNB) as a useful diagnostic tool in the pre-surgical management of patients with MCC and we also assessed the negative predictive value and false negative rate of combined cytological (US+FNAC) and histological (SLNB) procedure compared to the literature values obtained from different SLNB procedures.

Design: US examination was performed in 53 patients with diagnosed MCC and in 11 patients it was followed by FNAC of US suspicious lymph node. Smears were examined by routine cytological staining. Cases of uncertain diagnosis were stained in immunocytochemistry with a combination of anti-cytokeratin antibodies (CK 20, CAM 5.2).

Results: All FNAC were informative (10 LNs were positive for metastases, 1 was negative). Of all others 42 MCC cases, with nonsuspicious lymph nodes on US, in which no FNAC examination was performed, 6 carcinomas (14.3%) turned out to be lymph node positive on histological examination. One of the 11 (9.1%) negative cytological diagnoses was false negatives since lymph node metastasis of 5 mm of diameter was found by SLNB on histology. Based on these data, US+FNAC are endowed with

high sensitivity and accuracy (both of 90.9%) and absolute specificity (100%) and we suggest that US examination should be performed in all patients with MCC adding immunocytochemistry-supported FNAC only on US-suspect lymph node. Besides, the negative predictive value for our combined cytological and histological procedure was 94.7% (36/38) and the false negative rates in our series was 10.5% (2/19).
Conclusions: The present preoperative protocol (US+FNAC and SLNB) is reliable for screening patients with lymph node metastases, thus avoiding sentinel lymph node biopsy in 18% (10/53) of MCC cases.

539 The Utility of ATF3 in Cutaneous Epithelial Neoplasms

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Background: The histopathologic distinction between benign and malignant cutaneous keratinocytic proliferations can present a diagnostic challenge. Activating Transcription Factor 3 (ATF3) is a potential biomarker which may aid in the segregation of these lesions. ATF3 is a member of the ATF/cAMP response element-binding (CREB) transcription factor family, which functions as a downstream effector in the ras-mitogen activated protein kinase (MAPK) signaling cascade. ATF3 is typically induced by cellular stressors such as hypoxia and DNA damage; in addition its overexpression has been described in ductal carcinoma of the breast, prostatic adenocarcinoma, Hodgkin lymphoma and invasive squamous cell carcinoma (SCC). Furthermore, enforced overexpression of ATF3 in the oral mucosa of mice results in the development of squamous cell carcinomas. These findings suggest an oncogenic role for ATF3 in these tumor types, and we hypothesize that ATF3 expression may be a specific marker of SCC. Therefore, its expression could be used to distinguish SCCs from basal cell carcinomas (BCC) as well as benign keratinocytic lesions.
Design: In a pilot study, we characterized ATF3 expression by immunohistochemistry in a series of moderately differentiated cutaneous SCCs (n = 8), nodular BCCs (n = 8) and BCCs with squamous differentiation (n = 8). The percentage of cells with nuclear and/or nucleolar expression of ATF3 was scored (1 = <10% of cells, 2=10-50% of cells, and 3= >50% of cells) by at least two separate dermatopathologists.
Results: We demonstrate strong, nuclear and/or nucleolar expression of ATF3 in all cases of SCC (score=2 in 2/8 cases. 25%; score=3 in 6/8 cases, 75%) but either absent or focal, weak nucleolar ATF3 expression in nodular BCCs (score=2 in 2/8 cases, 25%). Interestingly, we identify strong nuclear and/or nucleolar positivity in BCCs containing areas of squamous differentiation (score=2 in 3/8 cases, 37.5%; score=3 in 1/8 cases, 12.5%).
Conclusions: We conclude that ATF3 distinguishes invasive SCCs from nodular BCCs and is a marker for squamous differentiation in invasive cutaneous carcinomas. In future experiments, we will interrogate whether the expression of ATF3 delineates SCCs and actinic keratoses from other benign keratinocytic proliferations and can therefore function as a useful and specific biomarker in equivocal cases.

540 Primary Cutaneous Langerhans Cell Sarcoma: A Report of Five Cases and Review of the Literature

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Background: Langerhans cell sarcoma (LCS) is a rare but potentially life-threatening neoplastic condition. The diagnosis of LCS requires morphological and immunophenotypic characterization to distinguish it from other malignancies.
Design: Five cases of LCS were encountered in the consultative practices of two of the authors.
Results: Two cases had a prior history of acute myelogenous leukemia or chronic myelomonocytic leukemia with blast transformation. The remaining patients presented initially with localized disease. In those with underlying leukemia, the eruptions occurred after initiation of chemotherapy. In all cases, the biopsies showed a sheet-like growth of atypical epithelioid cells with features of Langherhans cells by virtue of an eccentrically disposed reniform nucleus although with marked pleomorphism. In two cases, the close apposition of the tumor to the epidermis along with concomitant ulceration was initially thought to be consistent with an invasive melanoma. Phenotypic studies revealed a profile supportive of LCS including positivity for CD4, CD1a and S100 in all and variable staining for langerin lysozyme, CD83, CD31, and CD68. A PubMed literature search was also conducted using the keywords Langerhans cell sarcoma, malignant histiocytosis X, and indeterminate cell sarcoma. To the best of our knowledge, only 31 cases (including the present cases) of Langerhans cell sarcoma have been reported in the English-Language literature. Five (16%) of these were associated with an underlying myeloproliferative disease. Of the 29 patients for whom follow-up data was available, 14 (48%) died from disease, 12 (41%) achieved and remained in complete remission, 1 (4%) achieved a partial remission, and 2 (7%) lived with their disease. Only 2 of the 8 (25%) reported cases with only cutaneous involvement died.
Conclusions: Cutaneous Langerhans cell sarcoma represents a terminally differentiated myeloid tumor with a variable clinical course. The tumors can be confused with acute leukemia cutis and nonhematopoietic malignancies such as melanoma. Immunohistochemistry is of cardinal importance in establishing the correct diagnosis. A malignancy of terminally differentiated myeloid cells could imply a common stem cell defect hence explaining the potential association with underlying myeloproliferative disease.

541 PAX8 Is a Sensitive Marker for Primary and Metastatic Merkel Cell Carcinoma

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Background: PAX8, a nephric cell-lineage transcription factor initially characterized in renal cell carcinomas, is also well-recognized as a marker of Mullerian tract and thyroid tumors. From a previous tissue microarray study of non-renal neoplasms including a variety of skin tumors, we identified PAX8 staining in a small set of Merkel cell carcinomas, a finding not previously described. Herein, we explore PAX8 immunoreactivity in a larger series of whole-section primary and metastatic Merkel cell carcinomas to determine potential diagnostic utility.
Design: Whole-section slides from 25 Merkel cell carcinomas were stained with polyclonal PAX8 (pre-diluted, Cell Marque, Rocklin, CA) and two varieties of monoclonal PAX8 (prediluted clone MRQ-50, Cell Marque, Rocklin, CA; 1:100 dilution clone BC12, Biocare, Concord, CA). Nuclear staining intensity (I) and extent (E) was semi-quantitatively analyzed (range 0-3) with a comparison of staining thresholds required for a "positive" result (≥1+ vs ≥2+).
Results: The 25 Merkel cell carcinomas included 18 primary and 7 metastatic tumors from 17 males and 8 females (51-91 years, mean 75.6 years). 25/25 (100%) of cases showed positive polyclonal and monoclonal PAX8 staining with the Cell Marque antibodies (polyclonal: average I=2.4, average E=2.6, monoclonal: average I=2.0, average E=2.1). Utilizing a ≥2+ staining I and E requirement, 21/25 (84%) and 19/25 (76%) of cases were positive with the PAX8 antibodies (polyclonal and monoclonal, respectively) from Cell Marque. The Biocare monoclonal PAX8 antibody was negative in all cases.
Conclusions: PAX8 staining in Merkel cell carcinoma, a novel finding, expands the spectrum of tumors showing immunoreactivity for this marker. Both polyclonal/monoclonal PAX8 antibodies from Cell Marque show high (100%) sensitivity for Merkel cell carcinoma and may prove useful additions to a panel of diagnostic markers. Interestingly, the Biocare monoclonal PAX8 antibody does not show any staining of Merkel cell carcinoma. Both PAX8 antibodies from Cell Marque maintain strong sensitivity using a ≥2+ staining cut-point for a "positive" result (polyclonal>monoclonal at 84% vs 76%), an important finding in cases with scant tissue and/or background staining.

542 BRAF Mutational Epidemiology in Dysplastic Nevi: Does Different Solar UV Radiation Exposure Matter?

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Background: BRAF mutation rates have been reported in nevi and melanomas of homogeneous Caucasian cohorts. However, the demographics of BRAF mutations in dysplastic nevi (DN) of populations with differing solar UV radiation (UVR) exposure have not been investigated.
Design: Extended BRAF testing for 9 mutations in DN (n=125) from 101 patients derived from populations with differing UVR rates: 1) Lebanon (LB, n=68): UVR=110 kJ/m2/yr and Saudi Arabia (SA, n=57): UVR= 139 kJ/m2/yr was performed. Clinical data collected included age, sex, anatomic location and size. Histologic parameters including architectural and cytological features, as reported by Shea et al., in addition to solar elastosis grade, were recorded. Cumulative averages of UVR over a 21 year period were derived from The National Center for Atmospheric Research databases.
Results: BRAF mutation status (BMS) was obtained for 113 of 125 (9.6%; 12 failed PCR) cases resulting in an overall mutation rate of 63/113(55.8%). BRAF mutation rate differed significantly by UVR regions (LB 53.4%, SA 74.4%, $p<0.05$). V600E was the prominent mutation in 61/63 (96.8%) cases. A 6/15 (40%) discordant mutation rate was found in patients with multiple nevi examined including 2 patients with different mutation types. Histologic examination subdivided the dysplasia as follows; mild (n=24), moderate (n=60) and severe (n=41) with trunk predominance (72.8%). Higher rates of pigment in stratum corneum were identified in SA ($p<0.05$). The frequency of BRAF mutation showed marginal significant increase with advanced architectural and cytological atypia. Intermittent sun damage and compound nevus type were significant predictors of positive BMS ($p<0.05$). The histologic parameters predictive of negative BMS included upper extremity location, regression, cohesiveness and suprabasal melanocytes ($p<0.05$). Positive BMS was reasonably predicted by multivariate binary logistic regression [C-statistic (95% (C.I.)) = 0.72 (0.62-0.83)] by 2 independent predictors: 1) Geographic location [OR (95% C.I.) = 2.87 (1.14-7.21); p=0.025] and 2) Compound nevus type [OR (95% C.I.) = 4.52 (1.85-11.04); p=0.025].
Conclusions: In the Near Eastern cohort, there is a significant increase in DN BRAF mutation rate at higher UVR exposures supporting a role for UVR in promoting BRAF mutation. In patients with multiple DN examined, discordant BMS negates an underlying constitutional predilection.

543 Diagnosis of Melanocytic Skin Tumors by MALDI Imaging Mass Spectrometry (MALDI IMS)

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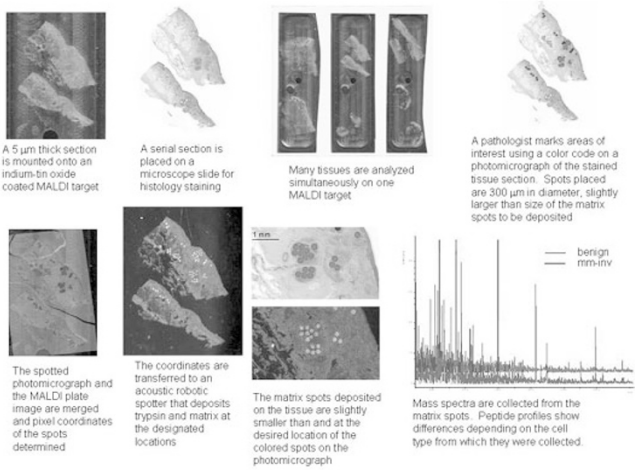
Background: Accurate detection of melanoma is an important factor in increasing survival rates of this fatal disease. Diagnosis of melanoma is still heavily based on histomorphology. Imaging mass spectrometry (IMS) combines the measurement capability of mass spectrometers with a surface sampling process that allows probing/mapping of the protein content of a sample. With MALDI, we used IMS for the discrimination of benign versus malignant melanocytic skin tumors.

Design: Formalin fixed, paraffin embedded (FFPE) tissue blocks of human melanocytic skin tumors were retrieved. Hematoxylin and eosin (H&E) and unstained sections were prepared. Digital microscope image of the H&E sections were annotated/superimposed to an image of the unstained section. Trypsin and matrix were spotted onto the unstained sections at the annotations. Custom geometry files were created to allow for the targeting of the specific areas on the tissue where trypsin and matrix have been applied and mass spectral profiles were collected using MALDI TOF MS.



A class prediction model was created using a support vector machine algorithm.

Results: 40 melanocytic skin tumors were selected, including 20 primary malignant melanomas (MM; mean age: 66 yrs) and 20 benign dermal nevi (DN; mean age: 54 yrs). We generated IMS data and profiled tissues using robotic matrix deposition techniques with a 200 µm resolution and identified 216 candidate peptide peaks (m/z range: 700-4500) which were preferentially over-expressed in MMs or in DNs ($p < 0.05$). When we used a genetic algorithm classification with 12 selected peptide peaks, we reached 91% spectral classification accuracy for MM and 100% for the DN class, with an overall spectral classification capability of 95% for the entire selected spots. Using a minimum of 5 X 200 µm spots per case, the accuracy in discrimination of benign versus malignant cases was 100%.

Conclusions: We compared the peptide expression profiles of benign versus malignant melanocytic skin tumors by MALDI IMS on FFPE tissues. With the minimum cutoff of 5 X 200 µm spots per case, we developed algorithms that discriminate these two entities with 100% accuracy.

544 The Expression of BRCA1-Associated Protein 1 (BAP1) in Dermal Melanoma

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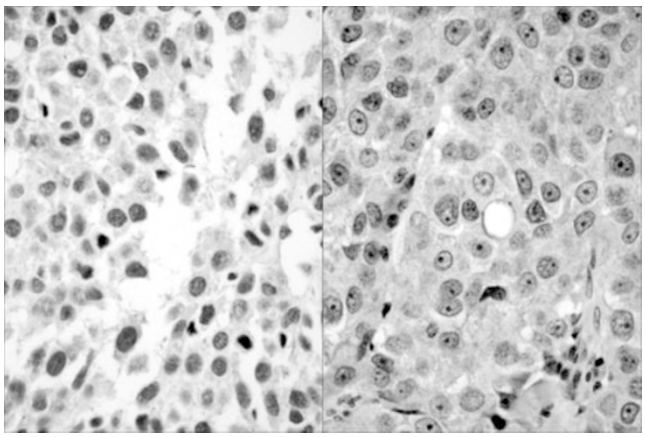
Background: BRCA1-Associated Protein 1 (BAP1) is a nuclear localized enzyme that belongs to the family of ubiquitin carboxy-terminal hydrolases. It is present on chromosome 3p21 in an area that has been shown to undergo both germline and somatic mutations. Recently, a number of kindreds known to develop both mesotheliomas and uveal melanomas have been shown to harbor germline mutations in *BAP1* as have sporadic mesotheliomas. While somatic mutations of BAP1 have been demonstrated in 5% of skin and mucosal melanomas, a detailed study of protein expression has not been conducted. Here, we show our results using BAP1 immunohistochemistry with a series of dermal melanomas.

Design: A tissue microarray was constructed using 42 metastatic melanomas (37 from dermal tumors, 1 from a mucosal tumor and 4 from unknown primary sites) from 22 men and 20 women with a mean age of 60 years (range 26-88). Immunohistochemistry was performed using the BAP1 antibody (Santa Cruz). Nuclear staining was scored as either positive or negative (complete absence of staining). Cytoplasmic staining intensity was assessed on a scale of 0 (no staining) to 3+ (diffuse strong positivity).

Results: Loss of nuclear staining was seen in 15 cases (36%). Cytoplasmic staining was intense in 33% of cases, moderate in 29% of cases and weak in 38% of cases. The figure shows intact nuclear staining (right) and loss of nuclear staining (left) with weak cytoplasmic staining.



Conclusions: In spite of the fact that somatic mutations of *BAP1* have only been observed in 5% non-uveal melanomas, 35% of our metastatic melanomas showed a loss of nuclear localization of the protein. This suggests that other factors may play some role in rendering BAP1 under-expressed or non-functional in dermal melanomas.

545 Epithelioid Malignant Schwannoma: A Clinicopathological Evaluation of 13 Cases

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Background: Epithelioid MPNST is an uncommon variant of MPNST. These tumors typically are composed of round cells with abundant cytoplasm with a prominent nucleolus. Unlike classical MPNST, which usually stains focally for S-100 protein, epithelioid MPNST or epithelioid malignant schwannoma stains diffusely for S-100 protein in 80% of cases.

Design: Thirteen cases reported as epithelioid MPNST were retrieved from the referral archives of two of the authors (E.C. and C.F.). The histology was reviewed by all of the authors and where immunohistochemical sections were unavailable for review, the results were taken from the original report. Clinical information and follow-up data were requested from the referring pathologists.

Results: Two patients had a history of neurofibromatosis type 1 (NF1). Sex incidence was essentially equal (7 males:6 females) and the majority (62%) of patients were older than 40 years. Six cases presented in the dermis and subcutaneous tissues (superficial), three cases were located in the subcutaneous tissue (deep), and two lesions were located in a deep major nerve. All cases were strongly positive for S-100 protein and negative for other melanocytic markers. EMA was focally positive in two cases and tumor cells were negative for other markers including desmin, smooth-muscle actin and pan-keratin. Six cases were entirely composed of epithelioid cells, while in five cases more than 60% of tumor cells had an epithelioid morphology. Only two cases were composed of a 50% population of spindle cells. In one case there was osteochondroid metaplasia and in a further case bone metaplasia was noted. In all cases tumor cells displayed cytologic atypia, a single prominent basophilic nucleolus and mitotic activity varied from less than one to 8 per mm square. Follow-up information was available in six cases. The latter range from 36 months to 84 months with a median of 54 months. Two patients died due to tumor spread and two patients had recurrence of the lesion. The other two patients are alive with no evidence of a recurrence or distant metastasis.

Conclusions: In conclusion, we report thirteen epithelioid malignant schwannomas with comparable findings to the literature of these lesions being less associated with NF1 patients, strong S-100 expression of the tumor, composition of the tumor with large epithelioid cells with a vesicular nucleus and prominent basophilic nucleolus, and increased mitotic activity.

546 Clinico-Pathological Correlates of Vulvar Melanosis with Melanocytic Atypia

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Background: Vulvar melanoma (VMM) is a rare malignancy of hair bearing and glabrous skin, which commonly denotes a poor prognosis. Early VMM in situ may not have a clearly defined precursor and is a well recognized clinical and histologic mimic of vulvar melanosis. Cases of vulvar melanosis with melanocytic atypia present a considerable diagnostic challenge.

Design: A 10 year retrospective search of our laboratory database was performed for cases of vulvar melanosis and VMM. All patients (pts) demonstrating melanocytic atypia and/or melanoma were subsequently longitudinally reviewed.

Results: Vulvar melanosis (Vm) was identified in 139 biopsies of 100 pts. Of those 14 pts originally demonstrated different degrees of cytologic atypia: mild (8), moderate (5) and severe (1). Two pts with high grade dysplasia (17 and 28 years old) were reclassified as atypical genital nevus on re-excision. Complete excision of the pigmented lesions without recurrences was achieved in the majority of the pts with high grade atypia (5 of 6) and in half of the pts with mild atypia. One pt had multiple excisions of lesions with moderate atypia during the course of 6 years without progression to melanoma. There were 13 pts with VMM (5 mucosal lentiginous, 4 superficial spreading, 2 nodular and 2 NOS types) ranging from 40 to 92 years of age (mean = 70 years). All VMM pts presented with either melanoma or at least severe melanocytic atypia on the initial

biopsy, shortly followed by a diagnosis of VMM. Vm with atypia was subsequently identified in 3 melanoma pts, demonstrating morphological features of de-novo intraepidermal epithelioid melanocytic atypia.
Conclusions: 1. Vm with mild atypia is not usually associated with either progression to the melanoma or lesional recurrence.
2. Rare cases of Vm with moderate atypia may present with multiple recurrences without progression to VMM, suggestive of field effect.
3. VMM appears to arise de-novo with no identifiable precursor lesion in the majority of cases.

547 Mass Spectrometric-Based Proteomic Analysis of Cutaneous Occlusive Vascular Diseases in Formalin-Fixed Tissues

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Background: Cutaneous occlusive vascular disease results from various etiologies. These intravasculuar thrombi are indistinguishable by light microscopy alone. As the management relies on treatment of the underlying disease, accurate classification of this disorder is of paramount importance. In this study, we evaluated various types of cutaneous occlusive vascular disease by laser microdissection and mass spectrometry based proteomic analysis.
Design: All available materials from 16 skin biopsies with intravascular thrombosis (7 calciphylaxis, 5 cryoglobulinemia, 1 leukocytoclastic vasculitis, 1 disseminated intravascular coagulation, 1 stasis ulcer, and 1 antiphospholipid syndrome) were retrieved. In each case, intravascular thrombi were identified under fluorescent light and microdissected. These fragments were digested into tryptic peptide and analyzed by nano-flow liquid chromatography electrospray tandem mass spectrometry (LC-MS/MS). The results were assigned peptide and protein probability scores, and they were displayed by the use of Scaffold program. Clinical information was obtained from our medical record system.
Results: Microscopically, all skin biopsies contained intravascular thrombi within the dermis and/or subcutaneous tissues. Secondary changes such as surface ulceration, dermal necrosis, RBC extravasation, and/or calcification (both intra and extravascular) were present in many of the biopsies. Proteomic analysis by LC-MS/MS was able to accurately identify specific proteins in all cases tested. Fibronogens were found in 9/16 cases (56%). In 4 of 5 cryoglobulinemia cases, the thrombi contained immunoglobulins (2 IgM and κ, 1 IgG and λ, 1 IgM, IgG, and κ) and the results were able to correlate with the bone marrow immunophenotypic data in patients with hematopoietic disorder. Interestingly, IgA, IgG, κ and λ were also detected in the intravascular thrombi of a patient with antiphospholipid syndrome.
Conclusions: LC-MS/MS based proteomic analysis of cutaneous intravascular thrombosis selectively identified immunoglobulin depositions in patients with cryoglobulinemia (both monoclonal and mixed). Detection of immunoglobulin constituents of the thrombi enhances our ability to further type cryoglobulinemia accurately independent of clinical information. Future study of additional cutaneous intravascular thrombosis in patients with other systemic diseases, should further clarify potential diagnostic roles for this novel application for cutaneous occlusive vascular diseases in formalin-fixed tissues.

548 Merkel Cell Carcinoma Immunoreactivity with Pax-5

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Background: Merkel cell carcinoma (MCC) is an uncommon high-grade neuroendocrine carcinoma of the skin. The differential diagnosis includes tumors with small round blue cell morphology. Pax-5 is a B cell specific transcription factor that plays an important role in B cell ontogeny and is commonly expressed in B cell non-Hodgkin's lymphoma, Hodgkin's lymphoma, alveolar rhabdomyosarcoma, and small cell carcinomas. The purpose of this study is to characterize the expression of Pax-5, using a monoclonal antibody of a clone SP34 different from those adopted in previous reports, and a panel of immunohistochemical markers in MCC patients.
Design: We used antibodies against Pax-5, terminal deoxynucleotidyl transferase (TdT), cytokeratin (CK) 7, CK20, thyroid transcription factor 1 (TTF-1), chromogranin and synaptophysin. Immunostaining was recorded semiquantitatively. Pax-5 nuclear staining was evaluated using a 3-tiered scale (weak, moderate, strong) and Pax-5 reactivity as follows: reactivity in fewer than 1% of tumor cells was scored as negative, reactivity in 1% to 25% tumor cells as 1+, reactivity in 26% to 50% tumor cells as 2+, and reactivity in more than 50% of tumor cells as 3+.
Results: Fifty-one MCC cases were reviewed. The cases occurred in 37 males and 14 females, ranging in age from 49 to 95 years (mean 75.9). Fourteen of the lesions were from the face, 10 from the trunk, 3 from the upper limbs, 17 from the lower limbs and 7 were metastases. Of 51 MCC cases, 34 (67%) were positive for Pax-5, showing, 1+ in 13/51 (25%), 2+ in 10/51 (20%), and 3+ in 11/51 (22%) tumor cells reactive. Nuclear staining intensity was weak in 20/51 (39%) and moderate in 14/51 (28%) MCC cases. No MCC (0%) demonstrated strong nuclear staining intensity for Pax-5. The immunohistochemical profile of 22 MCCs was further analyzed and demonstrated expression of TdT in 14/22 (64%), CK20 in 20/22 (91%), chromogranin in 16/22 (73%), and synaptophysin in 22/22 (100%). No MCC expressed CK7 (0%) or TTF-1 (0%). Of the 2 CK20 negative MCC cases, 1 case was positive for TdT, while both were positive for Pax-5.
Conclusions: The results indicate that Pax-5 is frequently expressed in MCC, showing on average 26% to 50% tumor cells reactive and moderate to weak nuclear staining intensity. Pax-5 may also be beneficial in rare cases of CK20-/TdT+ and CK20-/TdT-MCC. Pax-5 positivity in conjunction with CK20, TdT and neuroendocrine markers further supports the diagnosis of MCC when evaluating cutaneous small round blue cell tumors.

549 Fluorescence In Situ Hybridization (FISH) Reliably Distinguishes Tumour Cells of Benign Melanocytic Nevi from Those of Metastatic Melanoma

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Background: Malignant melanoma can be difficult to distinguish from a benign melanocytic lesion by histology. In previous studies, 3 chromosomal aberrations have been identified that are common in malignant melanoma but not found in benign nevi. Previous literature has been encouraging but not conclusive on the utility of fluorescence in situ hybridization (FISH) in assisting in diagnosis. In this study, we investigated the sensitivity and specificity of FISH to distinguish between benign nevi and metastatic melanomas to lymph nodes.
Design: Multicolour FISH was performed using a commercially available probeset (Abbott Laboratories, Abbott Park, IL), on formalin-fixed, paraffin-embedded tissue samples from 40 tumours: 20 benign melanocytic nevi, and 20 metastatic melanomas within lymph nodes, as determined by histologic assessment. Fluorescent signals for each probe were enumerated by 2 observers in 30 cells each per lesion. An algorithm using signal counts from a combination of 4 probes targeting chromosome 6p25 (containing RREB1 gene), 6 centromere (CEP6), 6q23 (containing MYB gene), and 11q13 (containing CCND1 gene) was used as suggested by the manufacturer. The following criteria were used: (1) the average CCND1 or MYB signals per nuclei is greater than or equal to 2.5 or (2) percent loss of MYB relative to CEP6 is greater than or equal to 31% or (3) the percentage of abnormal nuclei for RREB1 is greater than or equal to 63%. If at least one of the three criteria were met, the specimen was designated FISH positive. If none of the criteria were met, the specimen was designated as FISH negative.
Results: Of the 20 metastatic melanomas assessed, 18 were FISH positive. FISH detected significant abnormal nuclei for RREB1 in 17/20 cases (85%) and significant MYB loss in 12/20 cases (60%). Average signals per nuclei greater than 2.5 for CCND1 and MYB were present in only 7/20 (35%) and 4/20 (20%) cases respectively. All 20 benign nevi were FISH negative. Overall, the FISH test showed a sensitivity of 90% and specificity of 100%, in diagnosis of metastatic melanoma to lymph nodes.
Conclusions: These results provide further compelling evidence for the utility of multicolour FISH directed against 6p25 (RREB1), centromere 6, 6q23 (MYB), and 11q13 (CCND1), as an aid in determining malignant behaviour in melanocytic lesions.

550 Pathological Features of the Primary Melanomas of Patients Studied in the Multi-Center Sentinel Lymphadenectomy Trial-1 (MSLT-1) and Their Relationship to Sentinel Node Tumor Status and Clinical Outcome

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Background: The MSLT-1 (Multi-center Sentinel Lymphadenectomy Trial) was recently completed. We documented the demographics of trial patients and microscopic features of their primary tumors.
Design: 2001 trial patients were randomized into two groups. After exclusion criteria were applied, 260 patients were excluded and 28 patients were either ineligible or dropped out. The SNB group (n=1028) comprised patients who underwent wide local excision and sentinel node biopsy, followed by lymphadenectomy if the sentinel node was positive. The second group (n=685) were patients who underwent wide local excision, followed by clinical observation with lymphadenectomy when nodal metastases became clinically evident. We recorded: patient age, gender, primary site, Breslow thickness, Clark level, mitotic rate and histologic subtype of the tumor and the presence/absence of ulceration, lymph/vascular invasion, regression, peri/intra-tumor lymphocytic infiltrates, and microscopic satellites.
Results: In the SNB group, 189 patients had a positive SLN and 839 a negative SLN. In the SNB, node positive group, the mean age was 49.5 +/- 14.1 years. The commonest primary tumor sites were trunk 47.1% (n=89) and extremities 40.7% (n=77). Mean Breslow thickness was 3.14 +/- 2.05 mm with most tumors Clark level IV (63.5%). Lymphatic invasion was present in 25 patients (13.6%). The Cochran-Armitage trend test showed that the likelihood of a positive sentinel lymph node significantly correlated with number of risk factors: age <50, trunk primary, Breslow >2, Clark >III, and lymphatic invasion. Significantly, with 0 risk factors, the chance of positive lymph nodes is 6.6%, 1 risk factor, 11.6%, 2 risk factors 14.7%, 3 risk factors 28.5%, 4 risk factors 45.6% and 5 risk factors 80%.
Conclusions: The results of this study confirm our previous findings and data from the literature. Significant risk factors for sentinel node metastasis include patient age, lymphatic invasion, primary site, Breslow thickness and Clark level. The likelihood of a tumor positive sentinel node significantly correlates with the number of risk factors present. These findings are clinically relevant and indicate the need for nodal assessnent, only sentinel node biopsy can determine actual presence of nodal metastases.

551 Regulatory T-Cells in Alopecia Areata: New Evidence

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Background: Alopecia areata (AA) is believed to have an autoimmune mechanism in which the hair follicles are targeted by CD4+ and CD8+ lymphocytes. It has been proposed that the pathogenesis of AA shares similar pathogenesis with other autoimmune cutaneous diseases, such as vitiligo. Studies investigating the autoimmune mechanism of vitiligo have demonstrated that regulatory T-cells (Tregs) are a key component in cutaneous immune privilege. Almost all studies examining Tregs and AA have been

performed in mouse models. Our study compares the quantity of the CD25+/FoxP3+ Tregs in AA to the quantity of regulatory T-cells in folliculitis arising in non-autoimmune non-scarring alopecias in human specimens.
Design: A retrospective review of our electronic pathology database was performed to identify all cases of AA diagnosed by scalp biopsy (January 2002 through May 2011), and reviewed to confirm the original diagnosis. Additionally, 2 cases of folliculitis arising in non-autoimmune non-scarring alopecias were included for comparison. In the 9 cases of confirmed alopecia areata and 2 cases of folliculitis, immunohistochemical double staining for CD3/FoxP3, CD8/FoxP3 and CD25/FoxP3 were evaluated. Specifically, the number of CD25+/FoxP3+ cells was determined for each case by counting the total number of CD25+/FoxP3+ cells in 3 randomly chosen areas of peri-follicular inflammation at 40X. The CD3/FoxP3 stains were used to confirm the presence of T-cells and the CD8/FoxP3 stains were used to rule out CD8+/CD25+/FoxP3+ regulatory T-cells.
Results: Of the 9 AA cases and 2 folliculitis cases examined, 100% (11/11) demonstrated some degree of CD25+/FoxP3+ expression. The mean number of CD25+/FoxP3+ cells in the AA specimens was 2.9 cells (range 2 – 4 cells) and was significantly lower [p=0.0001 (two-tailed unpaired *t* test)] than the mean number of cells in the folliculitis cases (8.5 cells; range 8 – 9 cells). The CD3/FoxP3 stain demonstrated that the majority of peri-follicular inflammatory cells were CD3+, all FoxP3 positive cells coexpressed CD3, and rare scattered cells were CD3-. The CD8/FoxP3 stain demonstrated no cells that simultaneously stained for CD8 and FoxP3.
Conclusions: This study demonstrated that there is a significant decrease in CD25+/FoxP3+ Tregs in AA when compared to folliculitis arising within non-autoimmune non-scarring alopecias. These findings are similar to results from studies investigating Tregs in vitiligo. However, they are in contrast to published literature on AA in mouse models. Further studies in human subjects need to be examined in order to characterize the role of CD25+FoxP3+ Tregs in AA.

552 Clinical Correlates of Specific *BRAF* and *NRAS* Mutations in Melanoma

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Background: The treatment of melanoma is rapidly evolving due to an improved understanding of the molecular heterogeneity of this disease. While previous studies have identified differences between melanomas with activating *BRAF* and *NRAS* mutations, there is minimal information regarding characteristics that correlate with specific mutations of these genes.
Design: We reviewed the results of CLIA-certified molecular testing for *BRAF* and *NRAS* mutations performed, primarily using metastatic disease, on melanoma patients (pts) from 2/2007 to 9/2010. Pts with the following mutations were identified (n=437) for study: (1) *BRAF V600E* & *V600K*; (2) *NRAS* exon 1 (G12/G13) & exon 2 (Q60/Q61). Demographics (age, sex), primary tumor characteristics (subtype, location, histology, Breslow thickness, mitotic rate, ulceration), and characteristics at stage IV disease (age, BMI, serum LDH, involved sites, M category) were tabulated. The time from initial diagnosis to stage IV and overall survival (OS) were determined. Comparisons between mutation groups used Fisher's exact test for categorical variables and t-tests for continuous parameters. Kaplan-Meier (KM) analysis estimated the distribution of time to event endpoints; distribution comparisons used the log-rank test.
Results: Identified pts (n=437) with mutations were: *BRAF V600E* (n=230); *BRAF V600K* (n=72); *NRAS* exon 2 (n=111); *NRAS* exon 1 (n=24). Among pts with *BRAF* mutations, compared to *V600K*, *V600E* was associated with younger age ($p<0.0001$), female sex ($p=0.001$), primary tumor location ($p=0.008$), and longer interval from primary diagnosis to stage IV disease ($p=0.01$). Among pts with stage IV disease, *V600K* pts trended toward inferior OS among those who received treatment with a BRAF or MEK inhibitor ($p=0.28$) and those who did not ($p=0.21$), but statistical significance was not reached. Comparison of pts with *NRAS* exon 1 and exon 2 mutations identified significant associations with primary tumor location ($p=0.0004$) and histologic subtype ($p=0.02$). No other significant associations were identified.
Conclusions: Significant associations with pt demographics and primary tumor characteristics were identified with specific *BRAF* and *NRAS* mutations. As delivery of precision treatment and prognosis are increasingly guided by molecular features in melanoma, pathologists must remain fully cognizant of the clinical implications of molecular testing.

553 Loss of microRNA-205 Expression Is Associated with Melanoma Progression

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Background: Melanoma remains the most deadly of the common forms of skin cancer: ~8700 people died of melanoma in 2010, accounting for ~70% of all skin cancer related deaths. There is therefore a critical need to identify clinically informative biomarkers that illuminate those biochemical pathways central to the aggressive behavior of melanoma, since these will provide new targets for the design of rational therapeutic interventions. microRNAs (miRNAs) are short, non-coding RNAs that function in post-transcriptional gene regulatory pathways. Alterations in miRNA expression have been described in many different human tumors, and miRNAs function as key pathogenic components impacting cancer cell growth, survival and the capacity to metastasize. Moreover, it is feasible to obtain high-quality miRNA expression data from formalin-fixed paraffin embedded (FFPE) melanomas—often the only tissue available for retrospective analyses.
Design: We used archived FFPE specimens to define alterations in miRNA expression comparing nevi (n=10) to primary (n=10) to metastatic (n=10) melanomas using a microarray platform. We validated a discrete subset of changes in an independent set of nevi and melanomas by quantitative RT-PCR. Using melanoma cell lines, we characterized the function of one miRNA, miR-205, whose expression was progressively diminished from nevi to primary to metastatic melanomas.
Results: Enforced miR-205 expression in melanoma cells profoundly impairs cell motility and migration *in vitro* along with significantly decreased F-actin polymerization with only a modest reduction in cell proliferation. In an *in vivo* xenograft model, melanoma cells overexpressing miR-205 exhibit a reduced migratory capacity compared to control tumor cells. Mechanistically, miR-205 overexpression correlates with decreased expression of the zinc finger E-box binding homeobox 2 (ZEB2) mRNA and protein, and this coincides with increased expression of E-cadherin mRNA and protein.
Conclusions: Together, these results provide evidence for miR-205 as a suppressor of cell migration in the progression of malignant melanoma, and the dysregulation of miR-205 correlates with alterations in epithelial to mesenchymal transition pathways. The identification of differentially expressed miRNAs impacting melanoma cell biology will provide new potential therapeutic targets for the treatment of melanoma.

554 First Complete Full-Face Allograft Transplantation. Clinicopathologic Features of Graft Rejection

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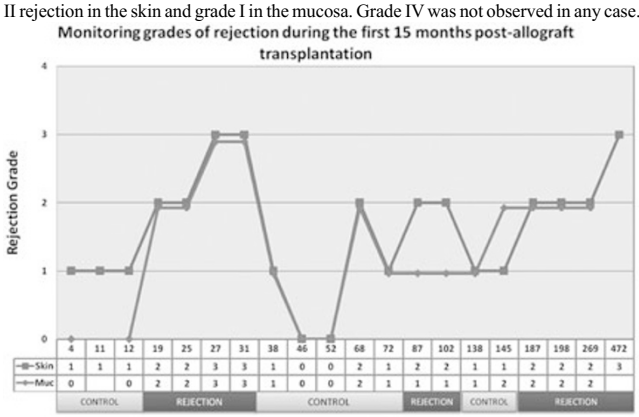
Background: The first human full face allograft was performed in Spain on March 27, 2010. The recipient was a 30 year old male with an accidental gunshot to face in 2005. We report the pathological findings from the skin and oral mucosa of this allograft during the the following 15 months after surgery.
Design: A total of 38 biopsies of skin (20) and mucosa (18) grafts were taken from 4 to 472 day post-grafting. All biopsies were analyzed, including those made by clinical suspicion of acute rejection (redness and edema of facial skin and oral mucosa) and control ones. All samples were examined following the criteria of Banff-Working system of classification of Skin-Containing Composite Tissue Allograft (CTA) proposed in 2008.
Results: Regarding the clinical evaluation, biopsies taken without clinical suspicious of rejection (control samples) (19) showed none rejection in 6 of 19 samples, mild rejection in 10 of 19 samples and moderate rejection in 3 of 19 samples. Skin control samples showed rejection changes more frequently than mucosa samples (7/10 vs 3/9). In contrast, when samples were taken due a clinical suspicion of acute rejection, histologically correspond to moderate grade (Grade II) in 12 of 19 cases, and severe rejection in 5 of 19 cases in both skin and mucosa samples. One clinical episode iniciated on day 87 post trasplant showed a discorrelation between skin and mucosa with a grade II rejection in the skin and grade I in the mucosa. Grade IV was not observed in any case.



Conclusions: Histopathological monitoring of full-face allograft trasplantation is mandatory to detect and grade acute rejection episodes with or without a clinical suspicious. The importance of histological grade I rejection without clinical manifestations remains unclear. Skin and mucosal biopsies showed a close correlation in histological assessment of acute rejection.

555 Microcystic Adnexal Carcinoma Versus Desmoplastic Trichoepithelioma: A Histologic and Immunohistochemical Comparison

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Background: Microcystic adnexal carcinoma (MAC) is a neoplasm with predilection for the head and neck and propensity for local recurrence, necessitating aggressive surgical treatment. Among the differential diagnoses for MAC is desmoplastic trichoepithelioma (dTE), another neoplasm with predilection for the head and neck, albeit with benign biologic behavior. Therefore, accurate diagnostic distinction between MAC and dTE is important in guiding clinical management. However, the histologic distinction between MAC and dTE can be challenging in the setting of a superficial biopsy. In addition, currently only one immunohistochemical stain, carcinoembryonic antigen (CEA), is reported as useful in distinguishing between MAC and dTE.
Design: We reviewed the histologic features of 30 cases of MAC and 39 cases of dTE, and performed CK19 immunostain on 20 MAC and 18 dTE. EGFR immunostain and FISH were done on 18 MAC cases.

Results: MAC cases occurred in older patients in comparison to dTE (median 67 years versus 34 years) and with a 20% recurrent rate. The head and neck was the most commonly involved site, 88% and 89% for MAC and dTE, respectively. In addition to histologic features previously reported as specific for MAC such as skeletal muscle and subcutaneous tissue invasion, perineural invasion, and glandular differentiation, we found the presence of cytologic atypia and mitotic figures to be significantly more frequent in MAC cases by Fisher's Exact test. In contrast, the presence of keratocysts, keratin granulomas and calcification was significantly more frequent in dTE cases ($p < 0.0001$). CK19 expression was seen in 70% (14/20) and 22% (4/18) of MAC and dTE cases, respectively ($p = 0.0044$). Strong membranous EGFR expression was noted in all 18 studied MAC cases. However, low polysomy of the EGFR gene was noted in only one MAC case.

Conclusions: The histologic features of keratocysts, keratin granulomas and calcification were significantly more frequent in dTE cases. In addition to features previously reported as specific for MAC, we found the presence of cytologic atypia and mitotic figures to be significantly more frequent in MAC cases. CK19 is a helpful adjunct since it is frequently positive in MAC cases. The role of EGFR inhibitor therapy in MAC cases with protein overexpression remains unclear. The lack of correlation between protein expression and polysomy/gene amplification suggests that molecular mechanisms other than gene amplification play a role in EGFR overexpression in MAC.

556 Cutaneous Myeloid Dendritic Cell Malignancies

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Background: Cutaneous dendritic cells neoplasms are exceedingly rare, and usually arise from plasmacytoid dendritic cells (pDCs). These neoplasms are categorized as blastic pDC neoplasms, also known as CD4+ CD56+ hematodermic malignancies. In contrast, myeloid dendritic cell (mDC) derived neoplasms represent a distinct type of hematodermic malignancy. The precedent literature regarding this entity is limited.

Design: Four cases of mDC malignancies were prospectively encountered in the routine and referral practices of one of the authors (CMM). The light microscopic studies were correlated with immunohistochemical results and clinical features.

Results: All patients were elderly ranging in age from 70 to 80 years of age (mean age 85 years); there were two males and two females. Each presented with a rapid onset skin rash. In each case the biopsies showed a noneffacing well differentiated small to intermediate sized monocytoid infiltrate with an unusual predilection to involve follicles, vessels, nerves, and the eccrine coil; there was accompanying attendant follicular mucinosis. The atypical monocytoid cells had a distinctive immunophenotype: they shared in common with the CD4+CD56+ hematodermic neoplasm positivity for CD4, CD56 and CD123. However at variance with the CD4+CD56+ hematodermic neoplasm was the expression amid the neoplastic cells for myeloid markers including CD68, CD11c, CD14 and lysozyme. In two cases there was peripheral blood chronic clonal monocytosis while in two patients the peripheral blood findings were more in keeping with acute myelogenous leukemia. Overall the findings were held to be consistent with a variably mature myeloid dendritic cell leukemia.

Conclusions: Cutaneous mDC neoplasms are clonal disorders of differentiated myeloid dendritic cells. The clinical settings are variable ranging from a chronic clonal monocytosis to one of acute myelogenous leukemia. Unlike the CD4+CD56+ hematodermic neoplasm, the clinical course ranges from a more indolent one, to an aggressive clinical course with death occurring shortly following the initial clinical presentation.

557 B7-H1 Expression in Merkel Cell Carcinoma and Co-Localization with Immune Infiltrates

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Background: B7-H1 expression by antigen presenting cells is a normal mechanism for inducing immune tolerance. Many tumor types have been shown to co-opt this mechanism, facilitating escape from immunosurveillance and therapeutic regimens. Immunotherapies for the blockade of B7-H1 and its receptor PD-1 are currently in clinical trials for patients with advanced malignancies, and objective tumor regressions have been observed. Notably, preliminary results suggest that B7-H1 expression by tumor cells correlates with the likelihood of tumor regression following PD-1 blockade. To determine whether patients with Merkel cell carcinoma (MCC) would be candidates for this emerging class of immunotherapeutics, MCCs were studied for B7-H1 expression. The relationship between B7-H1 expression and tumor infiltrating immune cells, including macrophages and tumor infiltrating lymphocytes (TILs) was also studied.

Design: Cases of MCC were identified in the surgical pathology archives. B7-H1 immunohistochemistry was performed using mAb 5H1 on archived, paraffin-embedded tissue, and cases demonstrating more than 5% membranous expression by tumor or associated immune infiltrates were considered positive. The degree of TILs was scored as mild (rare), moderate (focal), or severe (diffuse).

Results: We found that 56% (14/25) cases of MCC demonstrated B7-H1 expression by tumor or geographically associated immune infiltrates (median cell expression=5%). 100% (14/14) of the positive cases were associated with at least mild TILs, compared to 0% (0/11) of B7-H1 negative cases ($p<0.0001$). In all (14/14) positive cases, B7-H1 expression was observed solely at the interface with TILs, suggesting an adaptive immune response of B7-H1 expression as a result of T-cell secreted cytokines such as IFN-gamma.

Conclusions: B7-H1 is a critical immune modulating component within the tumor microenvironment. Paradoxically, while the presence of TILs suggests on ongoing host response, TILs may actually trigger their own downregulation by secreting cytokines that lead to upregulation of B7-H1 by tumor and associated antigen presenting cells. These findings, in combination with the studies that suggest that B7-H1 expression by tumor may serve as a biomarker of response, indicate that PD-1/B7-H1 blockade should be further explored as a therapeutic option in patients with metastatic MCC.

558 Desmin and CD34 Positivity in Cellular Benign Fibrous Histiocytoma: An Immunohistochemical Analysis of 100 Cases

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Background: Cellular benign fibrous histiocytoma (CBFH) represents a morphologic variant of cutaneous fibrous histiocytoma (FH) and accounts for about 5 percent of cases. Because of it relative monomorphism and fascicularity CBFH can easily be mistaken for a malignant lesion. In fact, while smooth muscle actin (SMA) positivity is common and a reassuring finding in CBFH, CD34, often used to distinguish CBFH from dermatofibrosarcoma protuberans (DFSP) can also be positive. To add to the confusion, desmin positivity may also be observed in a subset of CBFH. Desmin and CD34 positivity often seem to cause diagnostic difficulty and may lead to misdiagnosis. Our aim was to examine the immunohistochemical profile and incidence of desmin and CD34 expression in CBFH.

Design: 100 consecutive cases of morphologically typical CBFH were retrieved from consultation files. Clinicopathologic and immunohistochemical features were evaluated.

Results: Cytoplasmic SMA positivity was found in tumor cells in 93/100 cases (93%). Desmin positivity was found in 32% of the cases. Out of these 32 cases, 14 showed multifocal positivity, 9 showed focal positivity, 8 showed scattered cells, and 1 showed diffuse positivity for desmin. CD34 was positive in 6/100 cases (6%), with 1 being diffusely positive, 3 showing multifocal positivity and 2 showing focal positivity. More often there were entrapped CD34-positive dermal fibroblasts interdigitated with tumor at its periphery. There was no evident correlation between immunophenotype and anatomic site or other clinical variables.

Conclusions: Frequent desmin (32%) and occasional CD34 (6%) expression are encountered in CBFH. Desmin positivity can be explained on the basis of myofibroblastic differentiation in CBFH. The occasional CD34 positivity in a subset of CBFH should not be a deterrent from making the correct pathologic diagnosis, based on characteristic morphologic features.

559 Stathmin 1 Is a Potential Novel Oncogene in Malignant Melanoma

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Background: Stathmin-1 (STMN1) is thought to function by reorganizing microtubule in the cytoskeleton. In a recent high throughput study from our laboratory, we identified miR-193b as a potential tumor suppressor in melanoma (1). In that study, we reported that STMN1 was a potential target of miR-193b. Recently, experimental work in our laboratory has validated *STMN1* as a direct target of miR-193b. We found that STMN1 up-regulates cell proliferation and migration *in vitro*. Using a small tissue microarray (TMA), we demonstrated that STMN1 was up-regulated in malignant melanoma relative to benign nevi. In this study, we examined STMN1 expression in a larger, independent cohort, and assessed its role as a potential oncogene.

Design: From our archives, we assembled a cohort of 30 common benign nevi, 71 primary melanoma and 45 metastatic melanoma tissue samples, including 21 matched primary and metastatic melanoma tissues. From each case, two 6 mm cores were taken to construct the TMA. Subsequently, immunohistochemistry was performed using antibodies against STMN1. Staining intensity was quantified digitally using the Aperio Imagescope.

Results: The mean STMN1 staining intensity (total number of positive pixels/total number of pixels) in benign nevi, primary and metastatic melanoma was found to be 0.4%, 11.4% and 18.4% respectively, showing a statistically significant difference between benign nevi and primary melanoma ($P < 0.001$), as well as between benign nevi and metastatic melanoma ($P < 0.001$). The difference between primary and metastatic melanoma did not reach significance ($P=0.068$). In a sub-analysis using only the matched primary and metastatic melanoma pairs, the mean percentage positivity for primary and metastatic melanoma was 10.3% and 18.1%, respectively ($P = 0.10$).

Conclusions: In this study, we validate higher expression of STMN1 in melanoma relative to benign nevi, supporting the notion that STMN1 may function as an oncoprotein. Our data suggest that STMN1 may be involved in the early stages of melanoma pathogenesis since it is up-regulated in primary melanoma. Additional studies will be carried out to determine potential prognostic significance of STMN1 expression levels, and to provide more direct evidence for a role for STMN1 in melanoma pathogenesis using animal models.

1 Chen J, Feilotter HE, Paré GC, Zhang X, Pemberton JG, Garady C, Lai D, Yang X, Tron VA. MicroRNA-193b represses cell proliferation and regulates cyclin D1 in melanoma. *Am J Pathol.* 2010 May;176 (5):2520-9.

560 Contrasting BRAF Mutational Status and Solar UV Radiation Associations in Primary Versus Metastatic Melanoma

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Background: Melanoma has been viewed as a heterogeneous molecular entity for which solar UV radiation (UVR) and BRAF mutation status (BMS) are important determinants. We studied primary (PM) and metastatic (MM) melanomas from 2 UVR-distinct regions to elucidate the effect of prognostic predictors and UVR on BMS.

Design: Extended BRAF testing for 9 mutations was obtained for 95 PM [Lebanon (LB, n=55), Pakistan (PK, n=40)] and 65 MM [(LB, n=36), Pakistan (PK, n=29)] from 92 and 57 patients, respectively. Collected clinical data included age, size, gender and

anatomic location. Histologically, prognostic parameters and solar elastosis grade for PM were recorded. In MM multiple parameters including the site of metastasis, necrosis magnitude and degree of pigmentation were observed. Cumulative 21-year averages of UVR for LB (110 kJ/m2/yr) and PK (128 kJ/m2/yr) were derived from the National Center for Atmospheric Research databases.

Results: The overall BRAF mutation rate was 27.3% in PM and 56.9% in MM. V600E was the predominant mutation in 88% of PM and 92% of MM. A 3/9 (33.3%) discordant mutation rate was identified, 2 patients lost the mutation in the metastasis and 1 gained it. The relative incidence of BRAF mutation with UVR exposure was reversed for primary [PM (Low vs. High UVR): 5.4% vs. 43%] compared to metastatic [MM: 57% vs. 22%] melanomas ($p<0.05$). Predictors of BRAF mutation were trunk location and epithelioid cytology for PM versus subcutaneous metastasis and decreased pigmentation for MM ($p<0.05$). In PK, BRAF positivity was significantly more encountered in the absence of ulceration in PM and with decreased necrosis in MM. The other examined parameters did not affect the BMS in both PM and MM, irrespective of region. BRAF positive status in PM was reasonably predicted by multivariate binary logistic regression [C-statistic (95% confidence interval (C.I.) =0.67 (0.53-0.81) with two independent predictors 1) High UVR [odds ratio (OR) (95% C.I.) =14.947 (3.086-72.400); P=0.001] and 2) Trunk location OR (95% C.I.)= 3.646 (1.057-12.579); P= 0.041]. In MM, only high UVR [OR (95% C.I.)= 0.208 (0.063-0.0689); P=0.010] predicted BRAF mutation.

Conclusions: BMS in regions with different UVR exposures is reversed for PM as compared to MM. In view of newly invested targeted therapy, discordance in the BMS between PM and MM highlights the need for routine BRAF testing on both sites prior to treatment.

561 Perianal Verrucous Porokeratosis, a Rare Lesion Mimicking Inflammatory and Neoplastic Conditions

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Background: Porokeratosis (PK) represents a heterogenous group of lesions with disordered keratinization with a distinctive ridge-like border. The key histologic feature of PK is the cornoid lamella with a column of parakeratotic cells overlying epidermis with a decreased to absent granular layer and dyskeratotic cells in the stratum spinosum. A verrucous variant of PK involving the gluteal cleft has recently been described.

Design: To assess the frequency of perianal verrucous PK, we retrospectively searched our institutional pathology database from 2001-2011 for perianal skin lesions with one of the following diagnostic/microscopic terms, "porokeratosis", "hyperkeratosis", "cornoid lamella", "columnar parakeratosis", "atypical squamous proliferation", "verrucous" and "benign keratosis". Carcinomas, intraepithelial neoplastic lesions, melanocytic nevi, ulcers/abscesses/fistula tracts, and spongiotic dermatitides were excluded. Eighty-seven cases were available for review. The pre-biopsy clinical impression for each case was also collected.

Results: Of the 87 cases reviewed, four cases were identified as diagnostic of verrucous PK, and no misdiagnoses were found. The patients ranged in age from 50 to 75 (mean: 60; median: 58) and included 2 males and 2 females. The clinical impressions included inflamed seborrheic keratoses, malignant neoplasms, condyloma acuminata and inflammatory skin conditions, such as psoriasis, papular eczema, and lichen simplex chronicus. Histologically the four biopsies showed hyperkeratosis and acanthosis with cornoid lamellae overlying dyskeratotic cells, a decreased to absent granular layer and irregular papillomatosis. The remaining 83 patients ranged in age from 9 to 93 (mean: 44; median:45) and included 47 males and 36 females. The diagnoses included 5 (6%) psoriasis, 25 (30%) viral-associated keratoses and 53 (65%) variants of benign keratoses.

Conclusions: Gluteal verrucous PK is an uncommon variant of PK. In order to avoid a misdiagnosis, dermatologists and dermatopathologists must consider PK in the differential diagnosis of persistent verrucous, psoriasiform or lichenified perianal/gluteal plaques.

Education

562 The Frozen Section: Practicum Using Video Tutorial and Mock Specimens

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Background: Frozen section diagnosis for first year pathology Residents can be a daunting task because there is unfamiliarity with the equipment and procedure as well as the need to provide rapid, accurate diagnoses for the operating surgeons. As part of a new curriculum for incoming Residents, we introduced a new learning initiative for the frozen section process involving practice with fresh mammalian organs. This alleviated some of the trepidation involved before commencement of actual service work.

Design: The first step involved viewing of an instructional video detailing the entire process from specimen receipt to result reporting. This video was also made available online for further review at any time from any location. Fresh tissue was obtained as part of the autopsy orientation which involved the dissection of sheep and/or pig organ blocks obtained from a local meat packer. Each Resident was required to identify and embed 12 different tissue types including liver, spleen, kidney, heart, thymus, pancreas, esophagus/ stomach, adrenal, gonads, lung, bronchus, and lymph node. The use of the different tissue types allowed the resident to experience the different cutting characteristics exhibited by the divergent tissue types. Using proper technique involving the use of OCT embedding medium and the heat extractor, the trainee was shown several methods to obtain complete sections of the tissue. This allowed each Resident to develop the technique that worked best for him/her. After two proper sections were obtained using a cryostat microtome, the sections were fixed and stained using the standard H&E procedure. The sections were cover-slipped, and all cases were collectively reviewed, discussed and graded by the Residents and a group of Attending Pathologists. This allowed for recognition and analysis of various artifacts that can be present.

Results: Each trainee was assessed on his/her ability to obtain complete sections with proper staining patterns for each of the 12 tissue types. This exercise allowed the Residents to select the technique that worked best for them. The Resident also had time to become familiar with the frozen section equipment and procedure as well as tissue artifacts without the time and stress constraints of providing quick diagnoses to the operating room.

Conclusions: Residents exhibited increased confidence and proficiency with the frozen section procedures while handling actual patient diagnostic tissues during service work after completion of the frozen section practicum.

563 Utility of Digital Microscopy for Renal Biopsy Adequacy Assessment

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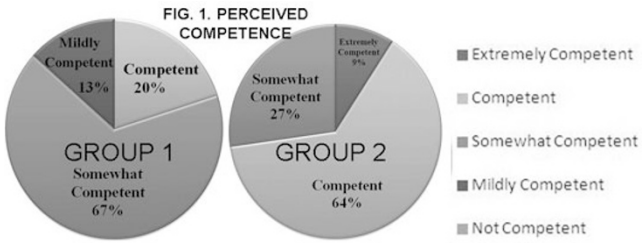
Background: At our institution pathology residents are required to have expertise in assessing renal biopsies for adequacy. We tested a novel approach, using a digital microscope connected to a netbook, for visualization of renal biopsy cores. We compared the glomerular counts with results from a conventional dissecting scope. We trained faculty, residents, and technical staff to use both methods. A survey of the participants was performed to assess the impact on operator competence and outcome.

Design: Digital and dissecting microscopes were used to assess renal biopsies. The specimens were visualized and a glomerular count was enumerated for each core including perfusing and obsolescent glomeruli. Both techniques were applied by two independent operators on a total of 23 biopsies and the results compared with counts from formalin fixed histologic sections. A survey questionnaire was distributed to individuals who operated the conventional method (Group 1) and those who used both methods (Group 2), to assess their impression of change in competence, professional relationship, and modality preference.

Results: The glomerular counts obtained by the two methods were compared to the counts obtained from histologic sections. This quantitative data demonstrated no significant difference in glomerular counts ($p = 0.5$) between the two methods, thus validating the digital microscope as a viable option for assessing adequacy. Qualitative data from the survey demonstrated a 9% increase in perceived level of competence and a 9% improvement in relationship with clinicians in Group 2.



Conclusions: A comparison of two methods of renal biopsy assessment demonstrated that the new digital microscope method yielded similar results to the conventional dissecting scope, thus offering a viable option. A survey qualitatively assessed subjective operator competence and its impact on professional relationships, revealing an increased level of perceived competence, and a more positive professional relationship with clinicians, in individuals who used the digital microscope.

564 Development and Validation of a Tool To Evaluate the Quality of Medical Education Websites in Pathology

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Background: The exponential use of the internet as a learning resource coupled with varied quality of many websites, lead to a need to identify suitable websites for teaching purposes.

Aim:

To develop and validate a tool which evaluates the quality of undergraduate medical educational websites; and apply it to the field of Pathology.

Design: A tool was devised through several steps of item generation, reduction, weightage and pilot testing. After developing a draft tool that encompasses criteria to be used in evaluating medical education websites, steps included pilot testing of the tool, post-pilot modification of the tool and validating the tool. Tool validation included measurement of inter-observer reliability; and generation of criterion related validity by testing the tool against a gold standard, construct related validity by measuring the relationship between the gold standard consensus with the actual score of the tool and the relationship of gold standard consensus with general website rating tools and content related validity by comparing the tool with general website rating tools and obtaining the subsequent gold standard rating of the tool. The validated tool was subsequently tested by applying it to a population of pathology websites.

Results: The tool was validated by applying a number of reliability and validity tests. Reliability testing showed a high internal consistency reliability (Cronbach's alpha = 0.92), high inter-observer reliability (Pearson's correlation r= 0.88), intraclass correlation coefficient = 0.85 and Kappa= 0.75. It showed high criterion related, construct related and content related validity. The tool showed moderately high concordance with the gold standard (Kappa=0.61); 92.2% Sensitivity, 67.8% Specificity, 75.6% positive predictive value and 88.9% Negative Predictive Value. The validated tool was applied to 278 websites; 29.9% were rated as recommended, 41.0% as recommended with caution and 29.1% as not recommended.