Table 1. Detection Rate for Metastatic Ovarian Cancer in Effusion Samples by PAX8 and WT1 Staining

		Number (%) of Positive Effusion Sample	
Surgical diagnosis of primary ovarian tumor	Total Cases	PAX8	WTI
High-grade SC	46	42 (91%)	42 (91%)
Low-grade SC	6	3 (50%)	6 (100%)
Clear cell carcinoma	5	5 (100%)	1 (20%)
Borderline mucinous neoplasm	1	0 (0%)	0 (0%)
MMMT	1	1 (100%)	1 (100%)
Carcinoma, mixed	9	6 (67%)	8 (89%)
Total	68	57 (84%)	58 (85%)

Conclusions: PAX8 and WT1 had similar overall detection rates for metastatic OC in effusion samples. PAX8 was more readily than WT1 to detect clear cell carcinoma but was less effective at detecting low-grade SC. Combining PAX8 and WT1 substantially increased the diagnostic accuracy. Both cell block sections and smears can be used for PAX8 and WT1 staining.

462 Urine Cytomorphology of Micropapillary Urothelial Carcinoma.

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Background: Micropapillary urothelial carcinoma (MPUC) is a rare variant of UC with an aggressive clinical course in terms of higher T stage, higher incidence of lymphovascular invasion, and higher incidence of lymph node and/or distant metastasis (our data not shown here). However, cytologic features of MPUC in urine cytology have not been well described. The aims of this study were to describe cytologic features of MPUC and compare them with those of high grade UC (HGUC).

Design: 21 urinary specimens (11 voided and 10 washings) from 14 patients with diagnosis of MPUC on follow-up surgical specimens, and 28 specimens (14 voided and 14 washings) from 28 patients with HGUC were retrieved. The cytologic features, single cell patter, papillary architecture, flat sheets/nests, 3 dimensional clusters, micropapillary (inside-out, acinar-like), nuclear grade, cytoplasm quantity, cytoplasmic vacuoles, and necrosis, were revaluated. Clinical follow-up was also reviewed.

Results:

Table 1. Cytologic features of MP-UC and HG-UC.

	Single Cells	Papillary architecture	Flat Sheets	3-Dimensional Clusters	Micro-papillary	Nuclear Grade	Cytoplasmic vacuoles
MP-UC	100%	47.6%	38.1%	85.7%	81.0%	2.7 ± 0.6	47.1%
HG-UC	100%	35.7%	35.7%	85.7%	14.3%	2.3 ± 0.7	14.3%
P Value	1.000	0.099	0.181	0.186	< 0.001	0.026	< 0.01

Chi Square test.

Table 2. Re-evaluation of urine specimens.

	MP-UC			HG-UC		
	Original	Re-evaluation	Possible MP	Original	Re-evaluation	Possible MP
UC	17/21 (81%)	20/21 (95%)	17/21 (81%)	13/28 (46%)	22/28 (79%)	4/28 (14%)
Suspicious for UC	1/21 (5%)		P < 0.001	2/28 (7%)	3/28 (11%)	
Atypical UC	1/21 (5%)	1/21 (5%)		6/28 (21%)	3/28 (11%)	
Negative	2/21 (10%)			7/28 (25%)		

Chi Square test.

8/14 (57%) MPUC and 1/28 (4%) HGUC showed metastasis to lymph node and/or distant organs (P < 0.001).

Conclusions: 1. On urine cytology, micropapillary architecture and cytoplasmic vacuoles are the two most useful features for the diagnosis of MPUC with sensitivity of 81%, specificity of 82%, positive predictive value of 77% and negative predictive value of 85%.

2. Nuclear grade of MPUC is slightly higher than that of HGUC.

3. MPUC can present with single cells, papillary architecture, flat sheets/nests, 3 dimensional clusters, and necrosis in urine cytology as seen in HGUC.

4. Patients with MPUC show higher incidence of lymph node and distal metastasis than HGUC (57% vs. 4%).

5. Careful evaluation of urine cytology for possible MPUC is very helpful to guide clinicians in choosing deep biopsy for possible MPUC cases, and predicting metastasis and prognosis.

Dermatopathology

463 Lymphatic Invasion in Melanocytic Tumors of Uncertain Malignant Potential May Predict Worse Outcomes.

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Background: Melanocytic tumors of uncertain malignant potential (MELTUMPs) are a subset of difficult melanocytic lesions that evade consensus from novice and expert alike. These lesions are bulky dermal melanocytic tumors that include atypical forms of spitzoid, cellular blue, and deep penetrating nevi, with features of both benign nevi and malignant melanoma. Previous studies of these lesions have shown that patients generally have favorable outcomes, with features such as mitotic activity, mitotic activity near the base, and inflammatory infiltration of the tumor as characteristics that may predict a more aggressive course. Lymphatic invasion has been found to a potential predictor of regionally metastatic disease in malignant melanoma. We attempt to test whether lymphatic invasion in MELTUMPs may be indicative of a worse prognosis.

Design: Fifty-five melanocytic lesions diagnosed as MELTUMPs at our institution and elsewhere were procured. Dual immunohistochemical staining for D240 and S100 is performed on the unstained tissue sections. Clinical follow up has been obtained for some of the patients.

Results: Of the thirty four cases of MELTUMPs currently stained, lymphatic invasion was found in 6 of them (6/34, 17.6%). Clinical history is currently obtained for 12 patients, with the current average clinical follow up being 10.9 years. Of these 12, four patients have clinical evidence of having a malignant course, and two of these patients have lymphatic invasion by dual immunohistochemistry (2/4, 50%).

Conclusions: Our preliminary data thus far shows that lymphatic invasion found by our method is quite promising as a potential tool to predict which MELTUMPs are more likely to develop a malignant course. Additional staining and clinical information gathering is currently ongoing to complete the study.

464 Deep Dermal Fungal Infections in Patients That Are Immunosuppressed.

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Background: Most clinicians use acute and granulomatous inflammation as a threshold for suspicion of an infectious organism. In immunosuppressed patients that are unable to mount an adequate immune response it has been postulated that the threshold for suspicion should be much lower. Over the years few journal articles have been published on characterizing fungal infections of the skin and the presentation in immunosuppressed patients. Most of these publications are case reports on opportunistic fungi such as *Fusarium*, melanized fungi, and *Cryptococcus*. Our goal is to look at a population of immunosuppressed patients and characterize the leading pathogens and compare the findings to those of immunocompetent patients in the literature.

Design: All the patients were selected from the Mayo database over the past 10 years. The inclusion criteria were that all patients will have had to have had a fungal infection of the skin that has been biopsied and also had a positive fungal culture. These patients will also have any kind of history that would lead to immunosuppression, whether it be by transplantation, treatment for an autoimmune condition, lymphoma, congenital/acquired immune condition, ect. All of the cases had a GMS (Grocott's Methanamine Silver) and/or PAS stain performed.

Results: Our initial results show that among 7 patients, *Alternaria* sp. was the most common organism affecting 3 patients. The remaining 4 patients were infected by *Exophilia jeanselmei*, *Scedosporium apoispermum*, *Fusarium* sp., and *Aspergillus* sp. A variety of host responses were observed ranging from a suppurative granulomatous response to a paucicellular immune response and mycetoma formation. The clinical presentations were predominantly nontender nodules that were biopsied to rule out carcinoma.

Conclusions: One of these patients had a large deep dermal nodular area with paucicellular lymphocytic infiltrate and necrotic debris that could be mistaken as infarct. Due to the patient's history, a GMS stain was ordered which revealed the entire area to be mycetoma which was initially very difficult to descern on H&E. This scenario could represent another diagnostic pitfall should there be low clinical suspicion.

465 Tyrosinase-Related Protein2 (trp2) Is a Melanocyte Differentiation Antigen (MDA) Useful for Surgical Pathology.

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Background: MDAs are expressed in cells and tumors of melanocytic lineage. MDAs such as gp100, Melan-A/MART1, and tyrosinase and their corresponding antibodies HMB45, A103, and T311 serve as important diagnostic tools in surgical pathology and are also employed as vaccine targets for the immunotherapy o malignant Melanoma (mM). However, little is known about trp2, another MDA, functionally a Dopachrome Tautomerase (DCT). In the present study, we determined the specificity of a novel anti-trp2 mAb clone C9 and analyzed the expression of trp2 in panels of normal and tumor tissue.

Design: MAb C9 to trp2 was obtained commercially. C9 was tested for specificity by Western Botting (WB) and ELSIA with rt-PCR tested cell lines and mM specimens. Its suitability for IHC was tested in in cell line pellets as well as in panels of normal and tumor tissues pre-typed by rt-PCR.

Results: In WB and ELISA, mAb C9 was reactive solely with trp2 mRNA-positive cell lines and tissues as well as the trp2 protein respectively but not with trp2-negative cell lines and not with unrelated proteins. In IHC, mAb C9 worked well in frozen and FFPE tissue employing antigen retrieval. In skin, typical staining of the melanocytes was present. Immunostaining of mAb C9 was completely inhibted by blocking with trp2 protein and not with other proteins. IHC of mM cell lines corresponded to the trp2 mRNA expression. 16/19 (84%) primary mMs and 19/33 (64%) metastatic mMs were C9 positive, both showing a mostly homogenous staining pattern. Several HMB45-negative cases, were trp2-positive. 10/10 mucosal mM were also trp2 positive. 6/6 desmoplastic mMs were negative. C9 reactivity was only focally present in only 2/9 angiomyolipomas. Besides melanocytes, no C9 reactivity was seen in normal tissues. Panels of non-melanocyic tumors such as carcinomas of colon, breast, lung, ovary, kidney and several sarcoma types were all C9-negative.

Conclusions: MDAs are important diagnostic tools as well as potential vaccine targets for the immunotherapy of mM. Here we show that mAb C9 is a novel specific marker for the detection of trp2. Expression of trp2 in mM parallels other MDAs such as Melan-A and tyrosinase being present in a high percentage of primary and metastatic mMs. Trp2 is a useful diagnostic marker and a potential vaccine target for melanoma.

466 Definition and Categorization of Combined Melanocytic Nevi.

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Background: "Combined nevus" is a term used to describe tumors composed of two or more distinct populations of melanocytes. While this term has historically been used to describe the combination of blue nevi with common nevi, it has more recently been applied to other combinations of benign melanocytic proliferations. We sought to

document the use of this terminology and the incidence and distribution of these tumors to propose consistent diagnostic nomenclature for practical use and comparison.
Design: The electronic records were reviewed for all cases that included both the terms "combined" and "nevus" in the diagnosis field between the years 2000-2010. The cases identified were then examined for diagnosis, presence of atypia, as well as age and sex of the patient.
Results: 514 combined nevi were identified, represented by four histologically distinct diagnostic categories: "common" junctional, dermal, compound or dysplastic nevus combined with 1) blue nevus, 2) Spitz or pigmented spindled cell nevus, 3) deep pigmented nevus (plexiform, deep penetrating, inverted type A), or 4) other combinations with or without a common nevus component. Of these nevi, 18% displayed cytological atypia, observed more often in combined common nevi with Spitz or deep pigmented elements (26/55, 47% and 23/97, 24% respectively) than in combined common and blue nevi (37/337, 11%). There was no difference in age distribution or gender between histological categories or in the banal versus atypical nevi.

Combined nevus histological and clinical distribution

Diagnosis	All cases	Inside cases	Consult cases	Age (mean)	Sex
Combined common and blue	337	321	16	4-86 (47)	179F:158M
Combined common and Spitz	55	50	5	7-76 (35)	34F:21M
Combined common and deep pigmented	97	84	13	4-79 (40)	58F:33M
Other combination	25	21	4	-	-

Conclusions: The term "combined nevus" may be applied to a range of melanocytic proliferations. The most common associations are with blue nevi, nevi with deep dermal pigmentation, and Spitz nevi, in decreasing prevalence. Atypia is more often associated with Spitz and deep pigmented tumors. Gender and age do not correlate with histological categorization or presence of atypia. Given the complicated melanocyte morphology of these unusual and sometimes atypical melanocytic tumors and the possibility of melanoma in the differential diagnosis, it is important to report the specific category of the melanocytic proliferation and the presence or absence of atypia in a consistent manner for adequate clinical follow up of the patient and further comparisons between patient specimens.

467 Epidermal and Dermal CD8+/CD3+ Ratios as Diagnostic Indicators of Mycosis Fungoides.

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Background: The diagnosis of mycosis fungoides (MF) presents a significant challenge in dermatopathology. In its early stages, the histopathologic features often overlap with inflammatory dermatoses. The differentiation of MF from reactive dermatoses requires several histologic and immunohistochemical criteria. The ratio of $CD8^+$ to $CD3^+$ lymphocytes in the epidermis and dermis has been suggested to aid in this evaluation. This study further assessed the utility of $CD8^+$ to $CD3^+$ T cell ratios to distinguish MF from inflammatory dermatoses in a larger case series than previously described.
Design: Forty cases with MF, as well as 20 control cases with inflammatory dermatoses were retrieved from our files and evaluated for immunohistochemical staining patterns for $CD8^+$ and $CD3^+$ T cells. The epidermal and dermal lymphocytic infiltrates were assessed separately by two methods. In the epidermis, $CD8^+$ as well $CD3^+$T cells were manually counted under high-power (40x objective) and the ratio was calculated. In the dermis, the $CD8^+/CD3^+$ T cell ratio was determined by visual estimate and grouped in 10% increments. All values were independently re-analyzed by two additional observers.
Results: A dermal lymphocytic infiltrate with intraepidermal lymphocytes was observed in all cases (60/60). Only one case in the MF group showed a complete absence of CD8 positive lymphocytes within the epidermis. Evaluation of the epidermis showed significantly lower $CD8^+/CD3^+$ ratios in the MF cases with an mean of 14% (SD=14). The control group exhibited a mean of 50% (SD=13). This contrast was less pronounced in the dermis with a mean of 15% (SD=9) in the MF cohort compared to 29% (SD=13) in the control group.
Conclusions: Our study confirms that evaluating the $CD8^+/CD3^+$ T cell ratio in the epidermis represents a useful tool to assist in the diagnosis of MF, while the diagnostic value of the $CD8^+/CD3^+$ ratio in the dermal component is ambiguous. Since rare cases in our series show some overlap, the epidermal $CD8^+/CD3^+$ T cell ratio should be used in conjunction with other existing histologic criteria for the diagnosis of MF.

468 Melanoma in Childhood and Adolescence: Clinicopathologic Analysis of 64 Cases.

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Background: The diagnosis of melanoma in children and adolescents is a significant challenge for both clinicians and pathologists due to the rarity of the disease, the often non-specific clinical presentation as well as the unusual histological features.
Design: 64 melanomas presenting before the 18th birthday were retrieved from the authors' consultation files. Hematoxylin and eosin-stained tissue sections as well as clinical and follow-up data were evaluated.
Results: Median age at presentation was 12 years (range: 2 months to 17 years) and there was a slight male predilection. No clinical pigment was evident in 11 tumors. The extremities were the most frequently affected site followed by the head and neck and trunk. 13 melanomas arose within a congenital nevus and 1 patient had a history of xeroderma pigmentosum. Histologically, median tumor thickness was 2.8 mm and the majority of tumors invaded at least into anatomic level IV (84%). The most frequent histological subtypes were Spitzoid (21) and nodular melanoma (21) followed by superficial spreading (12), nevoid (4), pigment synthesizing (3) and desmoplastic, neurotropic and acral lentiginous melanoma (1 each). Follow-up (median, 24 months) was available for 46 patients. Eighteen patients (39%) developed metastasis and 6 patients (13%) died from metastatic disease. Superficial spreading and nevoid subtypes occurred almost exclusively in adolescence (median: 16 years), only rarely affected the head and neck area and were associated with thinner tumor thickness (median: 1.0 and 1.1 mm). Nodular, Spitzoid and pigment synthesizing melanomas showed a wide age range (median: 11, 10 and 9 years) and anatomical distribution with deep tumor thickness (median: 4.0, 3.7 and 3.4 mm). Death from disease was observed only in patients with nodular melanoma.
Conclusions: This study further delineates the clinical and histological spectrum as well as clinical outcome of melanomas arising before the 18th birthday.

469 The Role of Immunohistochemical Markers in the Distinction of Epithelioid and Spindle Cell Tumors of the Skin: A Clinicopathological and Immunohistochemical Study.

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Background: Cutaneous epithelioid and spindle cell neoplasms occasionally pose a significant diagnostic challenge on purely histologic grounds alone. Given the substantial clinicopathologic overlap between these lesions, especially in small biopsies, the use of immunohistochemical (IHC) studies are essential. Conflicting reports exist about the degree of sensitivity of P63 and CD10 expression in carcinomas and atypical fibroxanthomas (AFX). Cytokeratin reactivity has been categorized as a sensitive marker for epithelial neoplasms; however, in poorly differentiated carcinomas its expression can be focal or even absent. In addition, tumors with different lineages of differentiation, such as leiomyosarcoma, may show focal expression of cytokeratin as well. The purpose of this study was to investigate the utility of a battery of IHC markers including podoplanin (D2-40), CD10, P63, SMA and cytokeratin, in distinguishing cutaneous epithelioid and spindle cell tumors.
Design: A total of 51 epithelioid and spindle cell tumors of the skin form the basis of our study, including 13 AFX, 13 spindle cell melanomas, 9 leiomyosarcomas, 7 leiomyomas, 6 angioleiomyomas and 3 sarcomatoid carcinomas. The antibodies employed were podoplanin, CD10, P63, wide spectrum cytokeratin and SMA. Appropriate positive and negative controls were run concurrently for the markers tested.
Results: IHC results are as follows- AFX: CD10 (11/13), P63 (0/13), CK (1/13), SMA (6/13), Podoplanin (6/13). Spindle cell melanoma: CD10 (8/13), P63 (0/13), CK (0/13), SMA (5/13), Podoplanin (2/13). Leiomyosarcoma: CD10 (4/9), P63 (0/9), CK (2/9), Podoplanin (1/9). Sarcomatoid carcinoma: CD10 (3/3), P63 (3/3), CK (2/3), SMA (3/3), Podoplanin (1/2). One case of leiomyoma was positive for Podoplanin; all other immunostains were negative in both leiomyoma and angioleiomyoma.
Conclusions: In summary, our findings show that the inclusion of certain IHC markers are a useful adjunct in the evaluation of epithelioid and spindle cell tumors of the skin. As opposed to other studies, we found that P63 is a highly sensitive marker for epithelial neoplasms. We also found that CD10 and podoplanin are non-specific markers in cutaneous epithelioid and spindle cell tumors of the skin regardless of their staining pattern. Awareness of this occurrence is extremely important because the prognostic and therapeutic implications of these neoplasms are quite different.

470 Can Primary Cutaneous Follicle Center Lymphomas (PCFCL) Transform/Progress to Primary Cutaneous Diffuse Large B-Cell Lymphomas (PCDLBCL)? An Immunohistochemical Study of 82 Cases.

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Background: PCFCL is characterized by a proliferation of neoplastic follicles in the skin and has an excellent prognosis with a 5-year survival of over 95%. PCFCL has several distinctive features when compared with nodal follicular lymphomas, as they are frequently negative for BCL2 and only less than 25% of the cases have a BCL2 rearrangement. The risk of transformation of PCFCL to PCDLBCL has not been clearly delineated in the literature. PCDLBCL as defined by WHO system, are composed of large, transformed B cells without follicular architecture. Transformation of systemic/nodal follicular lymphoma into aggressive diffuse large B-cell lymphoma is associated with rapid disease progression and poor prognosis. We studied 82 cases of PCDLBCL utilizing antibodies for follicular dendritic cells (FDCs), CD21 and CD35, to detect networks of FDC's that could indicate possible transformation of preexisting PCFCL to PCDLBCL.
Design: All cases were classified according to the new WHO/EORTC classification of cutaneous B-cell lymphomas. Primary skin involvement was defined as cutaneous lymphoma without nodal and/or visceral involvement after staging procedures. We included only cases that were characterized by diffuse infiltrates of B-cells without a follicular growth pattern. IHC staining was performed in all cases using CD21 and CD35 antibodies by a standard ABC method.
Results: Histologically, all the cases of PCDLBCL exhibited diffuse growth without evidence of a nodular pattern. Immunohistochemical studies were performed in all 82 cases and in 15 cases the presence of a CD21/CD35+ network of FDC's was noted throughout the tumor. Of the 15 cases that showed residual clusters of FDC's the tumors were located in the upper extremities (4 cases), leg (3), face (2), scalp (2), trunk (2), chest (1), and subclavicular area (1).
Conclusions: Systemic follicular lymphoma can transform or progress into DLBCL, which is usually associated with rapid disease progression, refractoriness to treatment and a poor outcome. This phenomenon, when occurring in the skin, has been rarely

addressed in the literature. In summary, these findings support that some cases of PCDLBCL may represent transformed PCFCL. It is important to be aware of this phenomenon since the transformation of an indolent cutaneous PCFCL into high-grade lymphoma may be associated with a more aggressive behavior.

471 Angioimmunoblastic T-Cell Lymphoma: Dermatopathological Features in a Series of 40 Cases.

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Background: Angioimmunoblastic T-cell lymphoma (AIL) is a systemic peripheral T-cell lymphoma with frequent skin involvement. Cutaneous manifestations are quite variable and frequently non-specific, the most common being a rash mimicking a drug eruption or a viral illness. Papulonodular, urticarial and purpuric lesions are also described. The dermatopathological features are correspondingly variable and in many reported cases they received little attention.

Design: Forty cases of cutaneous involvement by AIL were assessed dermatopathologically, with regard to the morphological and immunophenotypical features.

Results: Sixteen cases had a granulomatous pattern, varying from small granulomas to marked involvement suggestive of infection. Seventeen had a relatively non-specific superficial and deep perivascular infiltrate. In 16 cases there was often striking, usually localised, permeation of the eccrine glands, although syringotropism proper was uncommon. In only 7 cases were there prominent or easily identifiable eosinophils. In 18 cases cytological atypia was mild or minimal.

In most infiltrates the neoplastic cells had a peripheral CD4-positive T-cell phenotype. In 20/21 cases and 14/16 cases there were immunopositive cells for PD-1 and CXCL13, respectively, but these were almost always a minority, often just a few cells being positive. Similarly, CD10 was positive in 14/25 cases but in a substantial number of cells in only 2 cases. Epstein-Barr RNA (EBER) was positive in 12 of 30 cases tested, again usually in only a few cells. Four cases had a clearly malignant B-cell population. In at least 3 cases the cutaneous proliferation was of different lineage to the nodal neoplastic cells.

Conclusions: In cutaneous involvement by AIL, the diagnosis of lymphoma may be missed due to little atypia, the "non-specific" pattern of the infiltrate and/or conspicuous granulomas. Furthermore, in cases of evident lymphoma, the frequent absence of the typical AIL signature viz. eosinophil-rich CD10-positive EBER-positive infiltrate can lead to the correct diagnosis being overlooked.

472 P16 Expression Differentiates Merkel Cell Carcinoma.

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Background: Immunohistochemistry for p16 has proven useful in the diagnosis of viral driven epithelial malignancies including cervical carcinoma. In the case of cervical cancer, the E6 and E7 proteins of the Human Papilloma Virus (HPV) inactivate Rb and p53 which leads to aberrant cell proliferation and overexpression of p16. It is now known that Merkel Cell Polyomavirus plays a role in cell cycle dysregulation in Merkel cell carcinoma (MCC). To our knowledge, immunohistochemical analysis of p16 in Merkel cell carcinomas has not been investigated. We look to explore the expression of p16 in Merkel cell carcinoma. Additionally, we compare its sensitivity to an immunohistochemical stain for the protein expressed by the integrated Merkel Cell Polyomavirus (MCV) within tumor cells.

Design: Eleven Merkel cell carcinoma cases diagnosed at the VA San Diego Healthcare System over a 21 year period were examined for reactivity to p16 and MCV by immunohistochemistry.

Results: All eleven (100%) positive for p16 in selected tumor cells; however, only 66% of cases stained positive for MCV.

Conclusions: p16 expression in MCC suggests that the oncogenesis of the MCV derived carcinoma uses the inactivation of the Rb pathway similar to that of the HPV. p16 posivitity can be a useful diagnostic tool in differentiating merkel cell carcinoma from other small blue cell tumors. Additional studies to elucidate the incongruity between consistent p16 and variable MCV staining patterns are warranted.

473 Evaluation of Proliferation Index by Ki-67 and ProEx-C in Malignant Melanoma and Benign Melanocytic Nevi.

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Background: The correct classification of melanocytic lesions as benign nevi versus malignant melanoma is a high risk endeavor for patients and Pathologists alike. In diagnostically challenging cases, the proliferative rate of these lesions is considered a helpful distinguishing feature. The Ki-67 immunohistochemical proliferation index has been used to quantify proliferative activity in melanocytic lesions. ProEx-C is an immunohistochemical marker consisting of a cocktail of two antibodies that target minichromosome maintenance protein and topoisomerase II alpha protein, both of which are overexpressed in the nucleus during various phases of the cell cycle in proliferating cells. Moreover, ProEx-C has been shown to be a more sensitive marker of proliferation than Ki-67 in squamous lesions. Therefore, we hypothesize that ProEx-C will be a useful marker of proliferation in melanocytic lesions.

Design: We analyzed 199 melanocytic lesions (140 melanomas and 59 benign nevi) in a tissue microarray using an immunohistochemical method with antibodies directed against Ki-67 and ProEx-C. Melanocytes were counted at 400x magnification. Depending on the number of cells available in the core, 100 to 500 dermal melanocytes were counted (average per core: 307). Any positive staining within the nucleus was considered a positive cell.

Results: The average proliferative index of melanomas and benign nevi by Ki-67 staining was 16.9% and 0.3%, respectively ($p < 0.001$). By ProEx-C staining, the averages were 37% and 0.9%, respectively ($p < 0.001$). Comparison of primary melanomas with and without evidence of metastasis on follow-up did not show a statistically significant difference with either stain. However, the proliferative index by Ki-67 staining in primary melanomas (15.2%) was significantly different ($p < 0.05$) from that of metastatic melanomas (23.3%); statistically significant differentiation of these two groups by ProEx-C was not observed. Immunohistochemical proliferation rates for both markers paralleled mitotic activity.

Conclusions: ProEx-C staining index is a discriminating marker of proliferative activity in melanocytic lesions and may be useful in the diagnosis of melanoma and other difficult melanocytic lesions.

474 YouTube as a Public Educational and Consulting Tool in Dermatopathology.

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Background: According to the published literature "social media websites, such as YouTube, Facebook, MySpace, Twitter, and Second Life are rapidly emerging as popular sources of health information especially for teens and young adults," (Dermatology Clin. 2009 Apr;27(2):133-6, vi.) In this study, we surveyed the availability of YouTube videos dedicated to common dermatopathology diagnoses.

Design: Twenty-three separate search terms – representing the most common diagnoses likely encountered in a routine dermatopathology service – were entered into the "Search" section of the website, YouTube.com. In addition, the following terms were searched for: dermatopathology, mycosis fungoides, cutaneous lymphoma, and skin allergy. The number of videos uploaded for each term (uploaded by latest date Oct. 1, 2010) were compiled.

Results: Number of uploaded videos follows each diagnosis or search term: Psoriasis – 3, 760, Dermatofibroma – 10, Actinic keratosis – 108, Basal Cell Carcinoma – 143, Dermatopathology – 17, Melanoma 2, 760, Skin Allergy – 743, Nevus – 177, Atypical Nevus – 25, Squamous Cell Carcinoma Skin – 107, Paget's Disease Skin – 8, Neurofibroma Skin – 14, Contact Dermatitis – 221, Seborrheic Dermatitis – 50, Solar Lentigo 11, Atopic Dermatitis 323, Mycosis Fungoides – 8, Arthropod bite – 15, Eczema – 4020, Seborrheic Dermatitis – 193, Tinea – 225, Lichen Planus – 76, Granuloma Annulare – 3, Vasculitis – 128, Cutaneous Lymphoma – 23. dysplastic nevus – 6, Spitz nevus – 1.

Conclusions: Inflammatory dermatoses tended to have more videos uploaded by search term (example: eczema = 4,020 and psoriasis = 3,760) than skin cancers, perhaps reflecting the increased prevalence of such disorders in the youth and young adult population. "Melanoma" was the most common type of skin cancer found among the search terms (Melanoma = 2,760), likely due to the emphasis on public melanoma education by health care authorities (Online video-based patient education improves melanoma awareness: a randomized controlled trial. Telemed J E Health. 2009; Dec; 15(10):992-7). Some results were surprising such as the paucity of videos uploaded for Spitz nevus. Many of the videos were from university settings or private dermatopathology groups. Patients also uploaded videos. Some patient videos dealt with the emotional stress of a chronic dermatosis. The findings suggest that the popular video-sharing website is well-utilized as a patient educational tool. The medical community should not ignore this potentially important source of information (and misinformation).

475 Treatment of Psoriasis with Biologics Modulates the Expression of TNF-Alpha and Its Receptors.

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Background: The psoriasis is driven by activated T cells which release various cytokines that leading to proliferation and abnormal differentiation of keratinocytes. br]Elevated levels of TNF-alpha have been demonstrated in psoriatic skin lesions. TNF-alpha has a direct role in the development, proliferation, and maintenance of psoriatic plaques. It is a suitable drugs target for psoriasis treatment. Two distinct soluble TNF receptors have been identified, p55 (TNF-R1) and p75 (TNF-R2). We evaluated the clinical results, histopathological aspect and immunohistochemical expression of TNF-alpha and its receptors in skin biopsy before and after anti-TNF-alpha treatment.

Design: We have selected 40 patients with to moderate to severe psoriasis treated with anti-TNF-alpha biological drugs. Patients underwent skin biopsy at baseline and after 12 weeks. The skin biopsies were evaluated histologically and were tested the immunohistochemical expression of TNF-alpha, TNF-R1 and TNF-R2.

Results: We found high TNF-alpha, TNF-R1 and TNF-R2 expressions in early phase of psoriasis and in psoriasic lesions before treatment. The responsive patients were associate to presence in psoriasis lesions of ortocheratosis (p=0,04), proliferation of capillaries (p=0,01) and lymphocytic infiltrate (p=0,04) in dermal and with TNF-alpha endothelial expression (p=0,003).

Conclusions: TNF-alpha and its receptors expression change during the progression of plaque psoriasis. Anti-TNF-alpha therapy of psoriasis decrease the TNF-alpha and its receptors expressions. The morphology and cytokines expression in psoriasis could be helpful on deciding the treatment.

476 Clinicopathologic, Immunohistochemical and Molecular Analysis in the Differential Diagnosis of Reed Nevus and Spindle Cell Melanoma.

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Background: Reed nevus or pigmented spindle cell nevus, a variant of Spitz nevus, is a distinctive benign acquired lesion which mimics melanoma clinically and histologically. The main goal was to study a series of Reed nevi and spindle cell melanomas in order to elucidate useful features in their differential diagnosis.

Design: We collected 34 tumors, including 18 Reed nevi and 16 spindle cell melanomas. Clinical and histopathological features were reviewed. Using tissue microarrays, immunostains for HMB45, Ki67, cyclin D1, p53, $p27^{KIP1}$ and $p16^{INK4}$ were performed. In 21 cases (11 Reed nevi and 10 melanomas) we carried out fluorescence in situ hybridization (FISH) using probes targeting RREB1 (6p25), MYB (6q23), CEP6 (centromere 6) and CCND1 (11q13)(Abbott).

Results: In comparison with spindle cell melanomas (13 superficial spreading melanomas and 3 lentigo maligna type), Reed nevi presented in younger patients (mean 27 vs 61 years old, p<0.001), affected more frequently women (88% vs 50%, p=0.017) and predominantly presented in lower extremities while melanomas did in trunk and face. Reed nevi showed a smaller size (mean 3.6 vs 7.6 mm, p<0.001). Histological characteristics favouring the diagnosis of Reed nevus were: nests uniformity (p<0.001), symmetry (p<0.001), well-defined lateral demarcation (p=0.002), epidermal hyperplasia (p=0.005) and dermal melanophagia (p=0.002). Those favouring the diagnosis of melanoma were: lentiginous pattern (p=0.012), cytological atypia (p<0.001), high mitotic count (p<0.001), dermal regression (p<0.001) and elastosis (p<0.001). Conversely, we did not find significant differences in pagetoid or adnexal spread of tumor. Ki67 index was higher in melanomas than in Reed nevi (mean 11 vs 2%, p=0.009), whereas the rest of markers did not show significant differences. Five out of 11 Reed nevi (45%) were positive by FISH although they only met one criterion (gains in RREB1 greater than 29% of cells, but never higher than 40%). Conversely, all melanomas showed chromosomal alterations, most of them meeting 2 or more criteria.

Conclusions: Recently, ancillary techniques such as FISH have been developed in order to assist in the diagnosis of melanocytic lesions. However they must be assessed together with clinical and histological characteristics which are still essential for the differential diagnosis between Reed nevus and melanoma.

477 EMA Expression in Epithelioid Benign Fibrous Histiocytoma.

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Background: Epithelioid Benign Fibrous Histiocytoma (EBFH) is considered a morphologic variant of cutaneous FH, but lacks many characteristic features of ordinary FH and the epithelioid cytomorphology may mimic other dermal neoplasms. Our personal experience of EMA expression in some cases of EBFH has added to the diagnostic difficulty. The aim of this study was to examine the immunohistochemical profile and incidence of EMA expression in a series of EBFH.

Design: 44 cases of EBFH with available unstained slides were retrieved from consultation files. Clinical, pathologic and immunohistochemical features were evaluated.

Results: 26 patients were female and 18 male; median age was 39 years (range 7-82). 24 tumors arose on the lower limbs, 12 on the upper limbs, 4 in the head and neck, 3 on the trunk and 1 on the vulva. Most tumors were exophytic with a well-formed collarette and mild epidermal hyperplasia. All were well circumscribed (3 had a focally infiltrative edge) and composed of a monomorphic intradermal proliferation of plump ovoid cells with a sheet-like and/or storiform growth pattern, perivascular accentuation and varying numbers of admixed smaller spindled cells. Tumor cells had vesicular nuclei, small nucleoli and abundant palely eosinophilic cytoplasm. Binucleate cells were present in all cases and scattered multinucleate giant cells in 17/44 (39%). 2 cases showed prominent nuclear atypia. The tumor stroma was variably loosely collagenous or hyalinized and lacked a significant inflammatory infiltrate. Metaplastic ossification was seen in 2 cases and prominent myxoid change in 2 cases. Membranous EMA positivity was found in tumor cells in 27/42 cases (64%); 22 showed focal, multifocal or diffuse positivity and 5 had only scattered positive cells. There were no significant morphologic differences between cases that expressed and those that did not express EMA. Focal positivity for Factor XIIIa was found in 10/14 (71%) and D2-40 in 14/27 (52%). Scattered SMA positive tumor cells were seen in 11/43 (25%). Focal positivity for claudin was found in 3/42 (7%). CD163 staining highlighted stromal macrophages; however, in 5 cases it was difficult to exclude focal staining in tumor cells. Tumor cells were consistently negative for pan-keratin, S100, CD34, CD68, desmin, p63 and CD31.

Conclusions: The high incidence of EMA expression in EBFH is an unexpected finding and is a potential diagnostic pitfall. The significance of this finding is unclear, but raises the possibility that EBFH is unrelated to ordinary FH. Other immunophenotypic findings make perineurial, epithelial or myoepithelial differentiation unlikely.

478 Human Polyomaviruses 6 and 7 (HPyV6/7) Are Not Detectable in Merkel Cell Carcinoma.

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Background: Merkel cell carcinoma (MCC) is a rare cutaneous tumor that shows a high correlation with Merkel Cell Polyomavirus (MCPyV) in up to 80% of cases. However, there appears to be little difference in the morphology and molecular biology between MCPyV positive and negative MCC. An intriguing hypothesis has been that another similar polyomavirus may be associated with MCPyV negative MCC leading to a similar morphologic and molecular phenotype. Recently, two additional human polyomaviruses, Human Polyomaviruses 6 and 7 (HPyV6/7), were identified along with MCPyV in the skin of normal individuals by rolling circle amplification. Both HPyV6 and 7 share a high degree of sequence similarity to MCPyV. We sought to determine if the presence of HPyV6/7 could account for MCPyV negative MCC.

Design: DNA was extracted from formalin-fixed paraffin-embedded tissue blocks of 38 previously characterized MCC cases that included 22 MCPyV positive and 16 negative cases. A 110bp PCR product targeting the beta globin gene was used as an amplification control. Primers were designed to produce ~140bp products by targeting HPyV6 and 7 large T antigens and were aligned against all polyomaviruses in GenBank to ensure specificity. Cloned HPyV6 and 7 viral plasmids were used as positive controls. PCR for both HPyV6 and 7 was performed on all 38 cases and products detected by 2% agarose gel electrophoresis. To determine PCR sensitivity, a dilution series of viral plasmid DNA was constructed.

Results: The beta globin control gene was amplified in all 38 MCC cases. None (0/38) of the MCC cases, including both MCPyV positive and negative cases, had detectable HPyV6 or HPyV7. PCR sensitivity was estimated to be >5,000 viral copies.

Conclusions: While both MCPyV and HPyV6/7 are part of normal skin flora and show a high degree of similarity to MCPyV, we see no evidence of an association between HPyV6/7 and MCC. More importantly we see no evidence of HPyV6/7 in MCPyV negative MCC, arguing against the idea that another polyomavirus may be responsible for MCPyV negative MCC. While we cannot fully exclude the possibility of low level HPyV6/7 involvement in MCC, it seems unlikely that HPyV6/7 accounts for the pathogenicity of MCPyV negative MCC given that pathogenic MCPyV is present in very high copy numbers in MCPyV positive MCC.

479 Merkel Cell Carcinoma Expression Profiling Demonstrates No Significant Difference between MCPyV Positive and Negative Tumors.

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Background: Merkel Cell Carcinoma (MCC) is a rare and often fatal cutaneous malignancy that has been shown to harbor clonally-integrated Merkel Cell Polyomavirus (MCPyV) in up to 80% of cases. While morphologically identical, the molecular differences between MCPyV positive and negative MCCs are unknown. We used gene expression profiling to examine the differences between MCPyV positive and negative MCC and to compare the MCC expression profile to published data sets.

Design: Genomic DNA and total RNA were first extracted from formalin-fixed, paraffin-embedded tissue blocks. MCC cases were tested for the presence of MCPyV by PCR using the previously published MCVPS1 primer set. Cases that were MCPyV-positive were further tested by PCR viral-deletion mapping to confirm the presence of truncating large T antigen deletions. Next, a set of 10 MCC cases was selected, consisting of 6 MCPyV-positive and 4 MCPyV-negative MCCs. RNA from these cases was then amplified using the NuGen Ovation FFPE kit and run on Affymetrix Human Exome 1.0 ST arrays. The resulting expression data were clustered using Spotfire and the R statistical package. Confirmation of target genes was performed by TaqMan RT-PCR with 500ng of cDNA on a larger set of 16 MCPyV-positive and 12 negative MCCs.

Results: While there was little overall difference in expression between MCPyV positive and negative MCC, supervised hierarchical clustering defined a cluster of 7 genes with significant differences in expression including NF2, TSPAN15, CMYA5, CCDC55, NAPG, and two ESTs. However, TaqMan PCR for NF2, TSPAN15, CMYA5, and CCDC55 showed no significant difference between MCPyV positive and negative MCC in a larger set of 28 MCC cases. Non-supervised clustering of all MCC cases with published expression data including 158 normal tissues samples revealed an expression pattern most similar to mesoderm-derived tissues and not ectoderm-derived tissues.

Conclusions: Using array-based gene expression profiling from FFPE tissue, we could not detect a reproducible significant difference between MCPyV-positive and negative MCC in the 28 cases tested. This finding is consistent with the so-called 'hit and run' hypothesis in which MCPyV induces host DNA damage early in the pathogenesis of MCC but is not required for disease progression. In addition, the finding that MCC RNA expression profiles are most similar to mesodermal-derived tissue casts some doubt on the currently accepted histogenesis of MCC.

480 Interferon Regulatory Factor-4 Is Expressed Infrequently in Metastatic Melanomas and Correlates with Amplification of the IRF4 Locus.

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Background: Interferon regulatory factor-4 (IRF4) is a transcription factor expressed in melanocytes and some melanomas, but its function in melanocytic cells is unknown. The *IRF4* single nucleotide polymorphism allele, rs12203592*C, is a melanoma risk factor; this allele represses *IRF4* promoter activity, raising the possibility that IRF4 protects against melanoma. *IRF4* resides on 6p, a region often amplified in melanoma, suggesting IRF4 paradoxically might be up-regulated in advanced disease. We undertook this study to examine the relationship between IRF4 protein expression and *IRF4* copy number in metastatic melanoma.

Design: We performed IRF4 immunohistochemistry (MUM1p, Dako) in 116 cases of metastatic cutaneous melanoma (87 M, 29 F; mean age, 57 y). Expression was scored as: 0 = <10% staining; 1 = 10-30% staining; 2 = >30% staining, weak; and 3 = >30% staining, strong. *IRF4* amplification (>4 copies) or translocation was detected by fluorescence *in situ* hybridization using a breakapart probe. Associations with overall survival (OS) from time of biopsy were examined using the log-rank test. Metastatic melanoma cell lines were assayed for IRF4 expression (Western blot) and proliferation (EdU incorporation).

Results: Among tissue samples, IRF4 staining was seen in 25/116 (22%; 30% cutoff) or 38/116 (33%; 10% cutoff). *IRF4* amplification was seen in 47/116 (41%). The mean IRF4 staining score significantly correlated with *IRF4* gene amplification (0.93±1.00 *vs*

0.23±0.66 without amplification; p=0.00004, *t* test). One amplified case also had an *IRF4* translocation. Median OS was slightly longer for patients with IRF4 expression (63.0 *vs* 41.0 mos) or *IRF4* amplification (65.2 *vs* 41.9 mos), but these were not statistically significant. Cell line proliferation fractions were 92% in A375 (IRF4-negative), and 26% and 63% in C32TG and SK-MEL-28, respectively (IRF4-positive).

Conclusions: IRF4 is expressed in normal melanocytes, but, in contrast to one previous report, our data suggest IRF4 is expressed in only the minority of metastatic melanomas. IRF4 expression may have a protective effect in melanoma, becoming lost during melanoma development or progression. Indeed, proliferation fraction was highest in an IRF4-negative cell line. Paradoxically, IRF4 expression is associated with *IRF4* amplification in metastatic melanoma. Since 6p amplification is associated with poor prognosis in melanoma, our inability to identify significantly improved OS in patients with IRF4 expression may be due to the opposing deleterious effects of other amplified genes on 6p in these patients.

481 Sentinel Lymph Node Biopsies in Melanoma: A Retrospective Review of Outcomes and Tumor Burden.

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Background: Since the early 1990s, it has been seen that sentinel lymph node (SLN) biopsy for melanoma is a good predictor for involvement of the associated lymph node basin. As such, SLN dissection has been recommended for patients with clinically uninvolved nodal basins, with a primary lesion thickness greater than 1 mm. The purpose of this study was to correlate tumor burden in SLNs with clinical outcome.

Design: 99 patients from 1999 to 2008 with biopsy proven melanoma who also underwent sentinel lymph node biopsies were selected for this study. Melanoma sentinel lymph node protocol was performed which included immunohistochemical staining for S-100 protein, MART-1 and HMB-45. All skin biopsies were evaluated for traditional melanoma parameters.

Results: 65 patients had Breslow's tumor thickness of 1-4mm, and 8 had a tumor thickness of greater than 4mm. Also included in this study were 26 patients, with a tumor thickness under 1mm.

23 patients had positive sentinel lymph nodes (24%). The number of lymph nodes submitted ranged from 1-9. Isolated tumor cell (ITC) metastasis were identified in 6 patients, micrometastasis (MiM) in 9 patients, and metastasis (>2mm) were identified in 8 patients. Of the 6 ITCs, one had local recurrence and one had systemic failure. Of the 9 MiM, one had regional and systemic recurrence. Of the 8 patients with metastasis, six had systemic recurrence.

Of the 65 patients with a tumor thickness of 1-4mm, 17 had positive SLN (ITC: 5; MiM: 5; M: 7). Of the 8 patients with a tumor thickness greater than 4mm, 3 had positive SLN (ITC: 0; MiM: 2; M: 1). Of the 26 patients with a tumor thickness under 1 mm, 3 had positive SLN (ITC: 1; MiM: 1; M: 1). Seven patients had more then one lymph node positive, but did not correlate with Breslow's tumor thickness or tumor burden.

Conclusions: Sentinel lymph node biopsy is an effective method at predicting outcome in patients with melanoma. Breslow's tumor thickness did not correlate with metastatic tumor burden. Metastasis (>2mm) did correlate with increased local and systemic failure.

482 Expression of MiTF Can Distinguish Cellular Neurothekeoma from Plexiform Fibrohistiocytic Tumor.

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Background: Overlapping histopathologic features of cellular neurothekeoma (CNT) and plexiform fibrohistiocytic tumor (PFHT) predominantly composed of histiocytic cells, make distinction between these entities challenging. Some have suggested that CNT and PFHT are related entities. No prior study has demonstrated a reliable immunohistochemical panel to differentiate these entities.

Design: Cases diagnosed as CNT and PFHT, from 2004-2010 were retrieved from the pathology departments of Evanston Hospital, Cleveland Clinic and The University of Chicago with accompanying pathology reports. Each case was reviewed by at least two dermatopathologists for confirmation of diagnosis. All cases were stained for PAX2, NKIC3, CD10 and MiTF.

Results:

Immunohistochemical expression of cellular neurothekeoma and plexiform fibrohistiocytic tumor

Age	Sex	Site	Diagnosis	PAX2	NKIC3	CD10	MiTF
74	F	Left breast	CNT	3+	3+	3+	3+
38	M	Left thumb	CNT	-	3+	3+	3+
12	M	Right hip	CNT	3+	3+	3+	3+
25	M	Back	CNT	2+	3+	3+	3+
10	F	Left upper arm	CNT	2+	3+	3+	3+
31	M	Right scalp	CNT	3+	3+	3+	3+
12	M	Left shoulder	CNT	1+	3+	3+	3+
22	F	Right flank	CNT	2+	3+	3+	3+
UA	UA	Right neck	PFHT	3+	3+	3+	-
UA	UA	Left arm	PFHT	-	3+	3+	-
15	M	Posterior neck	PFHT	2+	3+	2+	-
5	M	Right forearm	PFHT	2+	3+	2+	-
48	F	Left neck	PFHT	3+	3+	3+	-

CNT = cellular neurothekeoma, PFHT = plexiform fibrohistiocytic tumor, 3+ > 75% positive expression; 2+ = 25-75% positive expression; 1+ < 25% positive expression; - = no expression; UA=unavailable

Conclusions: CNT and PFHT share many histopathologic features as well as IHC staining patterns. Of the stains we evaluated, we found that both CNT and PFHT were uniformly positive for NKIC3 and CD10 and both were frequently PAX2 positive. MiTF was strongly and diffusely positive in CNT and was consistently negative in the PFHT. Expression of MiTF may be a reliable marker for distinguishing CNT from PFHT predominantly composed of histiocytic cells.

483 Giant, Neglected Basal Cell Carcinomas and Neuropeptides: Could Alteration of Nociception and/or Mood Produce Neglect?

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Background: Infection and pain rarely complicate giant, neglected basal cell carcinomas (BCC). Notably, both normal and malignant epidermal tissues, including BCC, produce neuropeptides with opioid, antibacterial, and other effects. These peptides could potentially induce local analgesia and systemic effects such as mood alteration. Our objective was to document the clinicopathologic findings of a series of giant neglected BCC and compare the expression of neuropeptides to controls of conventional BCC.

Design: Clinicopathologic data was collected for 7 cases of giant (>5cm), neglected (>5years duration) BCC. The expression of serotonin (Abcam, MA), ACTH (Harbor-UCLA, Torrance, CA), ß-endorphin (Harbor-UCLA), met-enkephalin (Abcam) and neurofilament (Dako) was done by automated methods (Ventana Medical Systems Inc., Tucson, AZ). Clinicopathologic and immunohistochemistry findings were compared with 11 conventional BCC, matched for histologic pattern.

Results: Giant BCC occurred in 4 males/3 females with a mean age of 78yrs (range 62-90). All tumors had been hidden and neglected for greater than a mean 11yrs (range 5.1-20); were ulcerated; had a mean largest dimension of 17cm (range 5.5-30cm); affected the face and scalp (6) or forearm (1); and exhibited nodular and infiltrative patterns with bone invasion in 5 and destruction of orbit and eye in 2 patients. Compared to conventional BCC controls, giant BCC had a higher mean labeling index (LI) of expression of serotonin (2% vs. 0) and ACTH (8% vs. 4%), but a lower mean LI for ß-endorphin (40% vs. 78%) and met-enkephalin (30% vs. 76%). Neither controls nor giant BCC had intratumoral nerves, where as 46% and 17%, respectively had peritumoral nerve filaments.

Conclusions: BCC, conventional and giant, express proopiomelanocortin (POMC)-derived ß-endorphin and proenkephalin derived met-enkephalin with opioid activity at significantly higher levels than of POMC-derived ACTH or unrelated serotonin neuropeptides. Their dosage, possibly related to BCC size, could impact on the patients' pain perception and/or mood leading to neglect. Further investigates could include measurement of neuropeptides levels in the general circulation; examination for activated opioid receptors on sensory nerves in perilesional skin; and/or whether opiate antagonists affect the patient's perception of their tumors.

484 Cutaneous Pleomorphic Liposarcoma (PL): A Clinicopathologic Study of 33 Cases with Evaluation of MDM2 Gene Amplification in 19.

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Background: PL is a rare form of liposarcoma which rarely occurs in the skin where its behavior is incompletely defined. MDM2 gene amplification in a limited number of recent PLs suggests it may be part of the spectrum of well differentiated liposarcoma/dedifferentiated liposarcoma (WDL/DL) rather than a distinct subtype, but this finding has not been extended to a large group of tumors.

Design: PL involving dermis and/or subcutis were obtained from institutional and consultation files (n=33). Cases were evaluated with respect to age, sex, location (dermis [D], dermis and subcutis [DS], subcutis [S]), size, predominant pattern (pleomorphic spindled vs. epithelioid), amount of lipogenic differentiation, and tumor necrosis. MDM2 amplification was analyzed by FISH on formalin fixed paraffin embedded material in 19 cases.

Results: Patients ranged in age from 5-93 years (M:F=1.5:1). Tumors were located on the extremity (n=18), trunk (n=8), and head and neck (n=7) and involved the D (n=6), DS (n=9) and S (n=18). Tumor size ranged from 0.8 to 15 cm (median: 2 cm). All were mitotically active high grade sarcomas with either a pleomorphic spindled (n=26) or an epithelioid pattern (n=7) with a variable amount of lipogenic differentiation (<25% [n=15], 25%-50% [n=8], >50% [n=10]). Necrosis was present in some cases (n=5). MDM2 gene amplification was present in 3 of 19 cases. Follow up information in 20 cases (range= 1-192 mos; median= 52 mos; mean= 62 mos) disclosed local recurrences (2/20) but no metastasis or death from disease.

Conclusions: We conclude that 1. Cutaneous PLs, despite their high grade, have an excellent outcome attributed to their small size and superficial location. 2. The low incidence of MDM2 gene amplification indicates that not all PLs are related to WDL/DL. 3. PLs may, therefore, represent a group of sarcomas evolving by way of more than one molecular pathway.

485 Desmoplastic Melanoma: Expression of Epithelial-Mesenchymal Transition-Related Proteins.

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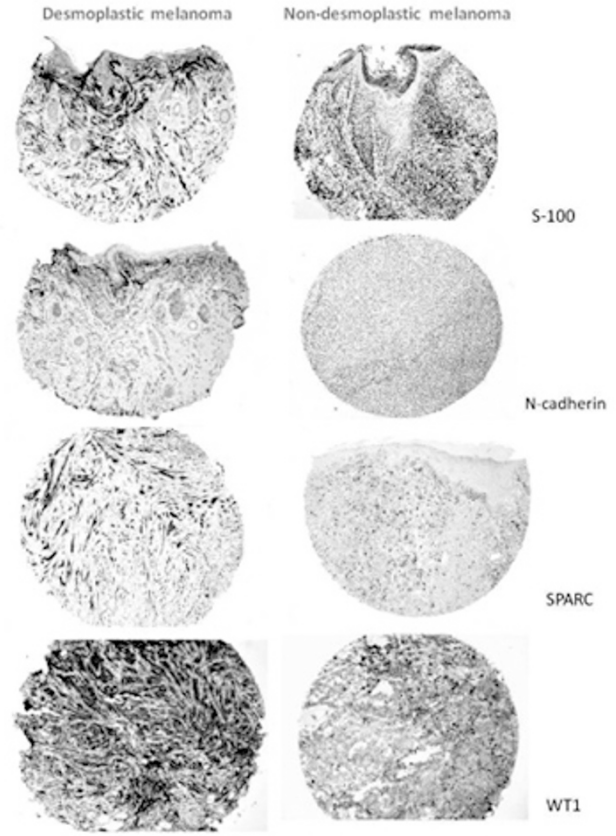
Background: Desmoplastic melanoma is a rare variant of melanoma, clinically ambiguous, appearing as an amelanotic, indurated plaque or mass in sun-damaged skin of elderly patients. Histologically, it's usually characterized by a poorly demarcated neoplasm composed by fusiform melanocytes dispersed in a prominent collagenous stroma, although a phenotypic heterogeneity is widely recognized. It often represents a diagnostic challenge, delaying its detection. Since a better prognosis seems to be

associated to this subtype of melanoma compared to conventional non-desmoplastic melanomas, refining its knowledge should lead to its improved and more effective management.
Design: To better understand the biological mechanisms implied in desmoplastic melanomas invasion, we analysed the expression profile of 29 (28 "pure" and 1 "combined") of desmoplastic melanomas. These data were compared to a series of 62 primary vertical growth phase non-desmoplastic melanomas using a set of proteins including melanocytic markers (S-100 protein and Melan-A) and epithelial-mesenchymal transition-related proteins (E-cadherin, N-cadherin, SPARC, WT1, and PKCα).
Results: The melanocytic origin of desmoplastic melanoma was confirmed by immunopositivity of the spindle cells for S-100. N-cadherin was highly expressed in desmoplastic melanomas (61%), while a weaker expression was observed in conventional melanomas (28%). In contrast, a lower positivity was showed in desmoplastic melanomas for E-cadherin (14%; $p<0.05$) compared with non-desmoplastic tumors (61%). SPARC) and WT1, previously well recognized to have an important role in local invasion have shown to be significantly overexpressed in desmoplastic melanomas (82% and 71% respectively) compared to conventional melanomas (43% and 47% respectively; $p<0.05$).



No statistically significant difference was demonstrated for PKCα.
Conclusions: The study demonstrates, for the first time, a prominent expression of epithelial-mesenchymal transition-related proteins in desmoplastic melanomas. However, the better prognosis attributable for desmoplastic versus non-desmoplastic melanomas of the same thickness remains to be explained.

486 Fluorescent In-Situ Hybridization (FISH) as a Tool To Assess for Chrosome Changes in Malignant Melanoma with Co-Existing Melanocytic Nevus.

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Background: Malignant melanomas are not uncommonly seen in association with pre-existing melanocytic nevi. However, whether the pre-existing nevi are precusor lesions or by-stander is not clear. Specific chromsome changes have been reported in malignant melanomas by FISH. In order to understand whether the pre-existing melanocytic nevi are precursor lesions, the chromosome changes in malignant melanoma and the pre-existing melanocytic nevi were evaluated by FISH.
Design: A total of 37 cases were retrieved from our database of patients with malignant melanoma. The main inclusion criteria was the presence of melanoma with co-existing melanocytic nevus. An algorithm-using signal counts from a combination of five probes targeting chromosome 6p25 (*RREB1*), 6q23 (*MYB*), 6 centromere and 11q13 (*CCND1*) with 11 centromere was used. A total of 50 cells in both the area of melanoma and nevus was counted. Benign and malignant areas were mapped out on the H&E sections. Criteria for a positive FISH result included (1) gain in *RREB1* relative to CEP6 greater than 55% or (2) gain in *RREB1* greater than 29%, (3) loss in *MYB* relative to CEP6 greater than 40% and (3) gain in *CCND1* greater than 38%. Analysis using SPSS 12.0 software was performed for t-test, Fisher test and chi-quare.
Results: The two most common types of melanoma included lentiginous type (n=11) and superficial spreading (n=19). Two cases of MM with spitzoid features with co-existing congenital nevi were also included. The malignant melanomas had a mean Breslow thickness of 0.61mm and most of them extended to the papillary-reticular dermal junction. FISH detected *RREB1* gain or *RREB1/CEP6* imbalance in 19/37 cases (51.7%), *MYB* loss in 12/37 cases (32.4%) and *CCND1/CEP11* imbalance in 22/37 cases (59.4%). 6 of 37 (16%) cases had no chromosomal gains or losses. Among the associated nevi, 1/37 (2.7%) had *RREB1* gains, 2/37 (5.4%) had *MYB* loss and 5/37 cases (13.5%) had *CCND1* gains. Compared to the benign part of the nevi, melanoma had genetic alterations more frequently (84 vs 16%, p=0.0001). The prevalence of coexisting genetic alterations in both lesions was 19%.
Conclusions: Concordant genetic gain/losses are relatively uncommon in melanomas with pre-existing melanocytic nevi (19%). This may indicate a diverse mechanism of precursor lesions for malignant melanomas and additional studies are warranted.

487 Anal Skin Tags with Granulomas: Association with Inflammatory Bowel Disease and Symptoms.

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Background: Anal skin tags are commonly inflamed, but rarely contain granulomas. This study was designed to assess the association of: 1. Granulomas in tags with inflammatory bowel disease (IBD), 2. Granulomas in tags with symptoms, and 3. Symptoms in tags with IBD.
Design: 373 anal tags excised at BIDMC from 1/1/97 to 3/1/10 were studied. Twenty were from patients with IBD (16 Crohn's, 3 ulcerative colitis, 1 indeterminate). Histology was evaluated for granulomas, and clinical record reviewed for symptoms (irritation, pain, discomfort, etc.).
Results: 1. Six of 20 (30%) tags from IBD patients and two of 353 (0.6%) tags from non-IBD patients contained tuberculoid granulomas (P=0.0001). Fourteen (70%) IBD patients did not have granulomas. 2. Five of eight (63%) tags with granulomas and 138 of 365 (38%) tags without granulomas were symptomatic (P=0.27**).** Five of 143 (3.5%) symptomatic tags and three of 230 (1.3%) asymptomatic tags had granulomas (P=0.27). 3. Fifteen of the 20 tags from patients with IBD were symptomatic (P=0.001). Fifteen of 143 (10%) symptomatic anal tags were from patients with IBD, in contrast to five of 230 (2.2%) asymptomatic anal tags coming from patients with IBD (P=0.001).
Conclusions: Granulomas in anal skin tags are highly correlated with IBD. 2. Granulomas in tags do not correlate with the presence of symptoms, however asymptomatic tags are unlikely to contain granulomas. 3. Having symptomatic tags does not correlate with having IBD, however 75% of IBD patients have symptomatic tags.

488 Differential Diagnosis of Melanomas Using Fluorescence In Situ Hybridization (FISH) – MelanoFISH.

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Background: Malignant melanoma is often difficult to distinguish from benign nevus. In this study, we investigated the utility of chromosomal anomalies in skin biopsy specimens to make this distinction by using multi-targeted fluorescence *in situ* hybridization (FISH).
Design: Skin biopsy specimens were retrospectively collected from patients with benign diagnoses (compound nevus, blue nevus, melanocytic nevus), dysplastic nevus (Clark's, compound, junctional, and residual), Spitz nevus, and melanoma. Each diagnosis was independently confirmed prior to study by two dermatopathologists. Unstained tissue sections were hybridized for 30 minutes using fluorescence-labeled oligo-DNA probes for chromosomes 6, 7, 11, and 20. Fluorescent signals for each chromosome were enumerated in 30 cells per case. A diagnostic criterion of gain in ten cells of any of these chromosomes or gains of any one chromosome in seven cells was considered positive.
Results: Evaluable FISH results were obtained in 465 cases. Numeric chromosomal anomalies which met the diagnostic criteria were found in 10% (20/205) of benign nevi, 5% (3/55) of dysplastic nevi, 39% (19/49) of Spitz nevi, and 72% (112/156) of melanomas. The mean number of cells with chromosomal changes was 3.4 for benign nevus, 2.8 for dysplastic nevus, 7.8 for Spitz nevus, and 13.8 for melanoma (melanoma vs. all other diagnoses, p<0.0001). The overall percent agreement between histologic diagnosis (melanoma vs. all others) and MelanoFISH results was 82%. The sensitivity = 72% (64-79%), specificity = 86% (82-90%). The area under the ROC curve was 0.79 (0.75-0.83), p < 0.0001.
Conclusions: Multi-targeted fluorescence *in situ* hybridization directed against chromosomes 6, 7, 11, and 20 can be useful for distinguishing melanoma from other melanocytic diagnoses. FISH appears less useful for distinguishing melanoma from Spitz nevus, an expected result given the known chromosomal anomalies in Spitz nevus similar to melanoma. Unlike other traditional non-oligo-based FISH probes, oligo-DNA probes required shorter hybridization time, allowing faster diagnostic evaluation.

489 Differential Expression of CK19, CD56 and CD57 in Neuroendocrine and Conventional Basal Cell Carcinoma: Is Neuroendocrine Basal Cell Carcinoma a Stand-Alone Entity?

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Background: Basal cell carcinoma (BCC) is the most common cutaneous neoplasm. Occasionally, Merkel cell carcinomas (MCC) display overlapping histologic features and may be confused with BCCs. Recently we have described a variant of BCC characterized by neuroendocrine (NE) histologic features (high N/C ratio, nuclear molding and a homogeneous chromatin pattern with inconspicuous nucleoli), increased mitotic rate and chromogranin positivity. We also found that Merkel cell polyomavirus (MCV) is frequently present in BCC with NE features. These findings raise the possibility of

a potential link between NE BCCs and MCCs. Our study aims to further define NE BCC, on a spectrum ranging from classic BCC to MCC, by investigating expression of NE markers CD56 and CD57 and the stem cell marker CK19 which are commonly expressed in MCC.

Design: A total of 37 BCC cases were selected including 19 with NE morphology (defined as above) and 18 classic BCC. All cases were also evaluated for histologic pattern, mitotic index/ mm2, presence of apoptosis, necrosis, squamous differentiation, ulceration and peripheral palisading. Immunohistochemical stains for CK19, CD56, and CD57 were performed in all cases. Stain was considered positive when >10% of the cells labeled. Results for CK20, chromogranin and synaptophysin were available from a prior study.

Results: The immunohistochemical results in classic vs. NE BCCs are summarized below.

	CK19	CD56	CD57	Chromo	Synapto	CK20
MCC†	100%*	96%	60%	95%	48%	92%*
Positive NE	37%	16%	0%	90%	5%	0%
Negative NE	50%	39%	0%	33%	0%	0%
P-value	*0.4*	*0.1*	NA	*0.0001*	*0.3*	NA

† Data from published literature
* *Perinuclear dot-like staining*

The expression of CD56 correlated with a lower mitotic rate (6 vs. 10 mitoses/ mm2 in CD56 positive vs. negative cases, respectively, p=0.05). There was no correlation between the other histologic features evaluated including histologic pattern, presence of apoptosis, necrosis, squamous differentiation, ulceration and peripheral palisading and status of the NE markers.

Conclusions: The expression rates of CK19, CD56 and CD57 are similar in NE and classic BCCs and much lower than in MCCs. These findings suggest that NE BCC has an "incomplete" neuroendocrine phenotype and is closer to classic BCC than to MCC. Yet, it differs from classic BCC by a higher rate of chromogranin expression and elevated mitotic rate and perhaps occupy an intermediate position between bona-fide cutaneous NE carcinomas and classic BCCs.

490 D2-40 Expression in Epithelioid Fibrous Histiocytoma.

JP Kapil, MS McLemore, VG Prieto. George Washington University Hospital, Washington, DC; UT – MD Anderson Cancer Center, Houston, TX.

Background: Epithelioid fibrous histiocytoma (EFH) is a variant of benign fibrous histiocytoma (BFH) that has a distinct histologic appearance but can be easily confused with other neoplasms, including melanocytic lesions. By immunohistochemistry (IHC), EFH is positive for factor XIIIa and CD68. D2-40, a lymphatic endothelial and dendritic cell marker, has been proposed as a very sensitive marker for standard BFH (100% of cases in a single series). However, no study has examined D2-40 expression specifically in EFH.

Design: For the present study, we searched the pathology files of our institution for cases diagnosed as epithelioid fibrous histiocytoma. From 2000-2010, there were nine cases with available slides. We performed IHC for D2-40, Factor XIIIa, CD68, CD163, and S100. Three pathologists reviewed the IHC results and quantified the percentage of epithelioid cells labeled and their intensity on a four-tier scale from 0 (negative) to 3+ (strong).

Results: D2-40 expression was seen in 8/9 cases, and in a majority of cases, greater than 40% of the epithelioid cells showed cytoplasmic and membranous labeling (See Table). Factor XIIIa expression was seen in 7/7 cases, in 5% to 60% of the epithelioid cells. CD68 expression was seen in 8/8 cases, greater than 60% of epithelioid cells in seven cases. CD163 expression was seen in 6/9 with 10% to 80% of epithelioid cells being positive. S100 was not expressed in any case.

DEMOGRAPHIC CHARACTERISTICS AND IMMUNHISTOCHEMISTRY RESULTS FOR OUR CASES OF EPITHELIOID FIBROUS HISTIOCYTOMA

#	Age (years), Sex	Site	D2-40*	FXIIIa*	CD68*	CD163*	S100*
1	68, M	right thigh	40%, 3+	40%, 3+	60%, 2+	-	-
2	40, M	right arm	10%, 1+	NA	NA	10%, 1+	NA
3	46, M	chest	-	20%, 1+	60%, 2+	-	-
4	38, F	external auditory canal	60%, 2+	20%, 1+	90%, 2+	80%, 3+	NA
5	44, M	right shoulder	70%, 2+	60%, 1+	80%, 1+	30%, 3+	NA
6	20, M	arm	90%, 1+	30%, 2+	60%, 2+	30%, 3+	-
7	20, M	nostril	90%, 1+	5%, 1+	10%, 2+	20%, 3+	-
8	24, F	nostril	80%, 2+	10%, 1+	80%, 2+	60%, 3+	-
9	18, F	right shoulder	90%, 3+	NA	100%, 3+	-	-

*% and intensity N/A not available

Conclusions: Our data indicate that EFHs, similar to standard BFHs, also express D2-40, albeit in a lower percentage of cases. Therefore, it seems that if immunohistochemistry is needed in cases difficult to diagnose, a panel of antibodies may be necessary, including D2-40, Factor XIIIa, and CD68.

491 Cutaneous Leishmaniasis in Lebanon, Syria and Saudi Arabia: Clinical, Histologic and Molecular Comparative Study of 122 Patients.

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Background: Cutaneous leishmaniasis (CL) is a growing problem in Lebanon (LB), Syria (SY) & Saudi Arabia (SA). The spectrum of manifestations of CL is broad both clinically & histologically.

Design: Skin biopsies from 122 patients with untreated CL from LB, SY & SA were analyzed. The clinical/histological features & molecular subspecies (MS) were compared. The clinical data collected included: age, sex, eruption type (papule: P; nodule: N & verrucous: V), duration, anatomic location (face: F; upper: U & lower: L extremity) & biopsy type. Histologically, multiple aspects were recorded including: Ridley's histological pattern & parasitic index system (PI), epidermal hyperplasia (EH) and elimination (EE), neurotropism (NT), granuloma type, interface/spongiotic changes, quantifying inflammatory cell composition & necrosis. To perform MS, PCR was performed using primers specific for the Leishmania ribosomal internal transcribed spacer 1 (ITS1-PCR) followed by restriction fragment length polymorphism (RFLP) analysis. The statistical analysis were performed using Chi square [1, 2] & ANOVA test.

Results: The species tropica was dominant in all regions (97-100%). The patients were of significantly younger age with shorter duration of the lesions in SY & SA compared to LB. SA cases showed more verrucous growth & EH. The mean PI was lower in cases from SA with less EE and NT. The PI showed statistically significant increase in facial lesions, nodular growth, EE and increase in plasma cells. The other clinical & histological variables were statistical insignificant.

Clinical and histologic features in different regions

Location	LB	SY	SA	p value
# of cases	40	51	31	
Male/female	42/58	63/37	65/35	0.09
Mean age (years)	42	29.6	29.2	0.01
Duration of lesion (months)	13.9	8.3	4.5	0.04
Anatomic location F/U/L	58/28/14	69/20/11	48/20/32	0.01
Eruption type P/N/V	8/68/22	0/100/0	3/54/43	0.1
Mean PI	3	3.1	1.3	0.3
Epidermal hyperplasia	23	27	42	0.01
Epidermal elimination	27.5	23.5	16	0.05
Neurotropism	55	45	32	0.2

All values represent percentage except when otherwise designated.

Conclusions: The pathogenesis of CL ivolves a multifactorial process where host response, region, PI, strain and other factors may play a role in the histologic and clinical presentation. Our data, despite the uniformity of the strain in all 3 regions, illustrates the histologic and clinical spectrum of CL.

492 Cutaneous Leishmaniasis Mimicking Other Inflammatory and Neoplastic Processes: A Study of 54 Cases.

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Background: Cutaneous leishmaniasis (CL) displays a considerable variation in the histological and clinical pattern. Clinically, CL presents as an erythematous papule that enlarges over the course of few weeks into painless ulcerated and crusted nodule/plaque. Histologically, the acute lesion displays sheets of histiocytes packed with amastigotes followed by granulomatous inflammation and scarring.

Design: After reviewing all the cases in our files (122 cases from 1992-2010) designated with the diagnosis of CL, 54 cases showed histological impression (HI) other then CL. Seven sections (4µm in thickness) from each formalin fixed paraffin embedded skin biopsy FFPE were stained: 3 H & E, 1 Giemsa, 1 AFB, 1 GMS, and 1 PAS. In cases with no unequivocal amastigotes identified, the diagnosis of CL was confirmed by PCR using primers specific for the Leishmania ribosomal internal transcribed spacer 1 (ITS1-PCR). The per-biopsy clinical diagnosis (PCD) of all 54 cases was collected.

Results: Out of the 54 cases, 20 cases presented with PCD other then CL. The PCD ranges from inflammatory dermatiditis to neoplastic processes and those included: deep fungal infection (DFI), sarcoidosis (SA), lupus (LE), mycobacterial infection (MI), Basal cell (BCC)/Squamous cell (SCC) carcinoma and lymphoma/pseudolymphoma (LY). The HI was recorded.

HI and PCD distribution.

	HI #	PCD #
SCC	16	2
BCC	0	1
LY	4	4
DF	1	0
DFI	11	5
FDE	1	0
SS	6	0
MI	4	3
PA	5	0
LP	1	0
SA	2	4
SASD	1	0
PLEVA	2	0
LE	0	1

PLEVA: pityriasis lichenoides; SS: secondary syphilis; LP: lichen planus; SASD: subacute pongiotic dermatitis; PA: paniculitis; FDE: fixed drug eruption; DF: dermatofibroma; LE: lupus.

Confirmation of the diagnosis by PCR was performed on 32 cases (58%) where no unequivocal amastigotes were identified.

Conclusions: The manifestations associated with CL are broad both clinically and histologically. Pathologist should be aware of such pitfalls especially in endemic areas.

493 Can Gene Expression Profiling Identify Primary Melanomas That Are Likely To Metastasize to Lymph Nodes?

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Background: Understanding of molecular mechanisms of metastasis in melanoma is limited, leading to ineffective treatment. Of primary melanomas, 20% spread to lymph nodes (LN) at time of biopsy and may benefit from sentinel lymph node (SLN) biopsy and completion LN dissection. The other 80% will not benefit and are at risk for surgery related morbidity. We propose to study primary cutaneous melanomas to identify gene expression signatures that distinguish primary melanomas likely to have spread to the regional (sentinel) LNs from those with a lesser risk of such metastases. We aim to separate patients likely to have SLN metastases for which immediate nodal surgery has potential benefits from the majority who, may be spared unnecessary nodal surgery.

Design: 10 primary melanomas were retrieved from UCLA FFPE block archives and cells grossly dissected. 5 cases were from SLN+ patients and remaining 5 from SLN- patients. Total mRNA was isolated, amplified and labeled using and Ambion Recover All ™ Total Nucleic Acid Isolation kit, Nu-Gen WT-Ovation ® FFPE RNA Amplification system and FL-Ovation ® cDNA Biotin Module V2, respectively. Samples were hybridized to the Affymetrix Gene Chip ® Human U133 Plus 2.0 Array. Data analysis was performed using Partek ® Genomics Suite Version 6.4. Differently expressed genes were selected at >= 2-fold and $p < 0.05$.

Results: Hierarchical clustering of the melanocytic lesions disclosed two distinct groups: 5 primary melanomas from SLN+ patients and 5 from SLN- patients. Gene expression analysis identified statistically significant genes that were differentially expressed. Most differences were associated with increased expression that correlated with lymphatic invasion. The reverse (decreased genes) was less frequent. Some genes that were relatively increased were biologically known to be involved in migration and invasion; PHPT1, TMEM163, ATP2A2, and ADAMTS10.

Conclusions: Differential gene expression patterns distinguished primary melanomas of SLN+ patients from SLN- patients. From a list of statistically significant genes, there was a trend of relatively increased expression that correlated with positive nodal status. This initial study show that critical genes are differentially expressed and may help identify genes responsible for lymphatic invasion. Furthermore, results shown may lead us to predict and identify which patients may need nodal sampling vs. those that may not based on the molecular profiling of primary biopsied lesion. Such advancements may contribute towards reduced unnecessary surgical procedures/morbidity, and overall medical costs.

494 Angiotropism in Congenital Melanocytic Nevi: Clinicopathological Study of 56 Cases.

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Background: Angiotropism has been reported anecdotally in congenital melanocytic nevi (CMN). Barnhill et al recently described medium-sized CMN demonstrating angiotropism of melanocytes about blood vessels (type non specified), and suggested that extravascular migration may be an important mechanism in the development of CMN. This study evaluates the presence or absence and type of angiotropism according to the size of CMN (giant, intermediate or small) in a pediatric tertiary care hospital between 2007 and 2009.

Design: We reviewed the charts of the CMN and only those in which the surface diameter was specified were included in this study: 25 small CMN (less than 1.5cm diameter), 20 intermediate CMN (between 1.5 and 20cm diameter)and 11 giant CMN (greater than 20cm diameter). The histopathology slides of these CMN were evaluated to assess the depth of CMN, the presence or absence of angiotropism, as well as the type of involved vessels in case of angiotropism. D2-40 was performed in all cases.

Results: Regardless of vessel-type, overall melanocytic angiotropism was observed in 36% in small CMN, 75% of intermediate CMN, and 100% of giant CMN. Refer to table 1 for a comparative view of specific vessel-type melanocytic angiotropism according to congenital nevus size.

Table 1: Specific vessel-type melanocytic angiotropism according to CMN size

CMN - size	lymphatic %	capillary %	venule/arteriole %
small, n=25	36	0	0
intermediate, n=22	70	20	5
giant, n=11	64	36	55

Conclusions: Our study showed that melanocyte angiotropism is a frequent finding in CMN. This frequency increases with the size of CMN; 36% in small CMN, 75% of intermediate CMN, and 100% of giant CMN. Moreover, the angiotropism involves lymphatics in small CMN, whereas capillaries, venules and arterioles are almost exclusively involved in intermediate and giant CMN. Melanocytic angiotropism is found in cutaneous venules and arterioles in 55% of giant CMN! These findings demonstrate the increased chance of angiotropism with the increased diameter size of CMN, support the theory of Barnhill et al suggesting that extravascular migration of neural crest stem cells may be an important mechanism for the development of CMN.

495 PAX-5 and TdT Expression in Merkel Cell Carcinoma and Pulmonary Small Cell Carcinoma: A Diagnostic Pitfall but Potentially Useful Discriminatory Marker.

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Background: Merkel cell carcinoma (MCC) is a high-grade neuroendocrine skin carcinoma characterized by cells with scant cytoplasm and fine, even chromatin. It is sometimes difficult to accurately diagnose by light microscopy due to its histologic similarity to other "small round blue cell tumors (SRBCTs)" including lymphoblastic lymphoma and small cell carcinoma. Immunohistochemical stains are often required to make a definitive diagnosis of MCC. PAX-5 is a B-cell specific transcription factor that is crucial for B-cell ontogeny and is detected in most B-cell lymphomas. Terminal deoxynucleotidyl transferase (TdT) is a DNA polymerase present in thymic T cells, B lymphoblastic leukemia/lymphoma (B-ALL), and some cases of acute myeloid leukemia. We had a recent case of MCC that expressed PAX-5 and TdT. We therefore sought to evaluate the expression of PAX-5 and TdT in MCC as well as pulmonary small cell carcinoma (PSCC) to determine if they were consistently expressed by these tumors.

Design: Paraffin blocks and slides from 28 MCCs and 10 PSCC were retrieved from the Pathology files of 3 institutions. PAX-5 and TdT immunohistochemical stains were performed and staining was graded as: negative (-), weak (1+), moderate (2+) or strong (3+). Stain distribution was graded as: focal (<10%), patchy (10-50%) or diffuse (>50%).

Results:

Positive Immunohistochemical Staining of PAX-5 and TdT in Merkel Cell Carcinoma and Pulmonary Small Cell Carcinoma

Tumor	PAX-5	TdT
MCC (n=28)	24 (86%)	22 (79%)
PSCC (n=10)	1 (10%)	9 (90%)

MCC: Merkel Cell Carcinoma; PSCC: Pulmonary small cell cacinoma, TdT: Terminal deoxynucleotidyl transferase

PAX-5 was positive in 24/28 (86%) MCC cases while TdT was positive in 22/28 cases (79%) MCCs. In PSCC, PAX-5 was positive in 1/10 cases (10%) and TdT in 9/10 cases (90%).

Conclusions: MCC and PSCC, which are both SRBCTs, coexpress the hematologic lymphoid markers PAX-5 and TdT. PAX-5 was expressed by the majority of MCCs but only rarely by PSCCs. TdT expression was frequent in both PSCC and MCC. Therefore, positivity for TdT and PAX-5 in any small round blue cell tumor should be interpreted with caution to prevent misdiagnosis as B-ALL. In cases where metastatic PSCC is to be differentiated from MCC, and TTF-1 is negative, PAX-5 may be a useful exclusionary stain since its negativity would favor a diagnosis of PSCC over MCC in this setting.

496 Comparing MicroRNA Expression Profiles in Dermatofibrosarcoma Protuberans and Dermatofibroma.

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Background: MicroRNAs (miRNAs) are small noncoding RNAs that are responsible for regulating gene expression in a variety of organisms including plants, animals, and protozoa. Expression profiles of miRNAs have been shown to differ between normal and tumor tissues, and between different tumor tissues as well. We hypothesized that miRNA expression is dysregulated in dermatofibrosarcoma protuberans (DFSP) when compared to dermatofibroma (DF).

Design: To address this, we compared miRNA expression profiles of four DFSP and four DF samples extracted from archival formalin fixed, paraffin embedded (FFPE) tissue using a miRNA array platform. This technique has been validated at our institution in a previous study (J Mol Diagn. 2008 Nov;10(6):513-9).

Results: Statistical analysis using unsupervised hierarchical clustering illustrated a clear difference in miRNA expression between the four DFSP samples when compared to the four DF samples. Supervised clustering revealed eight upregulated and nine downregulated miRNAs in DFSP relative to DF. In particular, miRNA-9 was upregulated 40 fold and miRNA-21 was downregulated 29 fold.

Conclusions: Our preliminary results suggest that miRNA expression profiles differ significantly between DFSP and DF, and may have an impact on its tumorigenesis. Further work will be done to validate our results of selected candidate miRNAs using quantitative RT-PCR and novel cloning and sequencing profiling techniques. As well, a miRNA expression profile for normal skin will be established as an additional control.

497 Epigenetics Markers in Acral Lentiginous/Mucosal Melanoma: Expression of EZH2.

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Background: Melanoma is a neoplasm with a complex pathogenesis and behavior. Molecular markers of outcome and the development of new therapies are needed. EZH2 is an enzyme involved in epigenetic changes through methylation of histones and participates in silencing cell cycle regulatory genes such as *INK4A* that encodes protein p16.

Epigenetic changes are important therapeutic targets, because they are potentially reversible. Acral lentiginous/mucosal melanomas (ALM/MuM) are rare variants of melanoma that likely evolve through molecular pathways different from the more common types of melanoma. The status of EZH2 has not been evaluated in ALM/MuM.

Design: Eighteen formalin- fixed, paraffin-embedded tissue blocks of melanoma from eighteen patients were retrieved from the files of UT-MD Anderson Cancer Center. The cases comprised: acral lentiginous melanoma (n=3); mucosal melanoma (n=7); superficial spreading melanoma (n=2); lentigo maligna (n=2); nodular melanoma (n=2); metastatic melanoma (n=2). Sections were obtained and stained. Immunohistochemistry for EZH2 (clone 6A10), p16 and MITF-1 was performed using standard avidin biotin methods. Percentage of area stained (< 10%, 10-50% and >50%) and intensity of staining (0 to 3+) were scored.

Results: Patients age ranged from 11 to 82 years old (mean age 60.6); the female to male ratio was 1:1. Sites of melanoma included: foot (n=3), conjunctiva (n=1), upper respiratory tract (n=2), rectal mucosa (n=3), urethra (n=1), back (n=1), neck (n=1), thigh (n=1), ear (n=1), lymph node (n=1), skin metastasis (n=1), head (n=1) and arm (n=1).

Nuclear expression of EZH2 was observed in 53.3% of all melanoma cases (8/18). 66.6% (6/9) cases of ALM/MuM showed strong expression of EZH2. All ALM/MuM co-expressed p16 and MITF-1 by immunohistochemistry.
Conclusions: In our series, EZH2 was expressed in most cases of ALM/MuM. This novel marker is a potential target for therapy. To the best of our knowledge, this is the first time that the expression of EZH2 is assessed in ALM/MuM.

498 Immunoglobulin-A Associated Small Vessel Vasculitis: A Ten-Year Experience at the Massachusetts General Hospital.

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Background: Leukocytoclastic vasculitis (LCV) together with vascular IgA deposition on direct immunofluorescence (DIF) are not pathognomonic of Henoch-Schonlein purpura (HSP), as a previous community-based series has shown that infection was often the cause of non-HSP IgA vasculitis (Am J Dermatopathol 1999;21:234-240).
Design: We performed a retrospective analysis to determine the strength of association of IgA vascular deposition with HSP. 67 patients with a histologic diagnosis of LCV and accompanying DIF studies were identified between 3/2000 and 7/2010. All cases exhibited leukocytoclasia, fibrinoid necrosis of the vessel walls, and erythrocyte extravasation. Of those, 31 cases had vascular IgA deposition on DIF examination and 27/31 had available clinical information.
Results: Using the American College of Rheumatology 1990 classification criteria for HSP, which requires at least two of four criteria (age ≤ 20, palpable purpura, abdominal pain, histologic evidence of LCV) in order to distinguish HSP from other forms of vasculitis; 23/27 (85%) met criteria for HSP. Two criteria were met in 65% (15/23), three criteria in 26% (6/23), and all four criteria in 9% (2/23). 20/23 patients (87%) had palpable purpura, 13/23 (57%) had renal manifestations, 9/23 (39%) had abdominal pain, 8/23 (35%) had arthralgias, and 2/23 (9%) were ≤ 20 years old. Out of the remaining non-HSP yet IgA+ cases (15%) where the etiology was apparent, half were due to drug hypersensitivity (2/4).
Conclusions: The presence of IgA deposition on DIF is not pathognomonic of HSP since only 85% of IgA+ small-vessel vasculitis was clinically diagnosed as HSP in this series. Our findings showed that in a hospital-based population such as ours, non-HSP IgA+ vasculitis was most commonly due to drug hypersensitivity.

499 Target Marker for Immunotherapy in Metastatic Melanomas of Rare Morphology Subtypes.

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Background: Metastatic melanoma can be a challenging diagnosis with its diversified morphology. Metastatic melanomas with rhabdoid or desmoplastic features are rare but should be included in the differential diagnoses when evaluating tumors with rhabdoid morphology or desmoplasia. Immunohistochemical staining profiling of such tumor is not only important for correct diagnosis but also critical for potential application of the adoptive cell transfer immunotherapy. By using gene-modified lymphocytes targeting a specific tumor marker, adoptive cell transfer has been shown to be a highly effective treatment for metastatic malignant melanoma. In this study, with 6 different antibodies, we characterize the expression of potential immunotherapy target markers for melanomas of rare morphology subtypes.
Design: We analyzed the expression of Melan A/MART-1, HMB45, tyrosinase, S100, KBA.62 and NY-ESO-1 by immunohistochemistry on 8 metastatic melanoma with rhabdoid features and 5 metastatic melanomas with desmoplastic changes collected between April 2008 and April 2009. Five primary melanoma lesions corresponding to the metastatic rhabdoid lesions are available for review and are concurrently examined.
Results: In our series, conventional melanoma markers including Melan A/MART-1, HMB45, Tyrosinase have high sensitivities in detecting metastatic melanomas with rhabdoid features (S100:100%, Melan A/MART-1:100%, HMB45:75%, Tyrosinase:88.9%) but low sensitivity in detecting melanomas with desmoplastic features (S100:100%, MART-1:20%, HMB45:20%, Tyrosinase:40%). The relatively new melanoma marker KBA.62 stains positive in all the rhabdoid and desmoplastic lesions (sensitivity:100%). NY-ESO-1, a cancer-testis antigen and an ideal marker for adoptive cell transfer immunotherapy, is expressed in 37.5% of the metastatic melanoma lesions with rhabdoid features (n=8) and 50% of the desmoplastic lesions examined (n=4). Intriguingly, none of the 5 primary lesions for the metastatic rhabdoid specimens have recognizable rhabdoid morphology.
Conclusions: Our results indicate that melanomas with rhabdoid features can be accurately diagnosed with conventional and novel melanoma markers though desmoplastic lesions are usually only positive for S100 and KBA.62. NY-ESO-1 is expressed in 37.5-50% of the lesions examined, which provides histologic evidence for using this specific adoptive cell transfer therapy with relatively small side effect in these patients. The metastatic rhabdoid melanomas do not have rhabdoid morphology in their primary lesions in our series.

500 The Spectrum of Merkel Cell Polyomavirus Expression in a Variety of Cutaneous Neoplasms and in Neuroendocrine Carcinomas from Different Anatomic Sites.

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Background: Most Merkel cell carcinomas (MCC) display pure neuroendocrine differentiation (PMCC) while a minority show combined neuroendocrine and non-neuroendocrine elements (CMCC). The identification of Merkel cell polyomavirus (MCV) in a majority of MCCs has suggested a viral-induced oncogenesis. Recent work has shown lack of MCV expression in CMCCs in contrast to findings in PMCCs. Moreover, studies of non-MCC tumours such as pulmonary neuroendocrine tumours (NET) and cutaneous basal cell and squamous cell carcinomas (SCC) have also yielded negative results. Our objective was to further explore the prevalence of MCV expression in a variety of different tumours.
Design: We studied 120 cases of MCC (24 PMCC, 13 CMCC, 13 MCC metastases) and non-MCC tumours of cutaneous (10 benign follicular tumours, 15 SCCs, 15 melanomas) and non-cutaneous sites (15 pulmonary NET, 15 gastrointestinal NET) diagnosed at our institution between 1989 and 2010. MCV expression by immunohistochemistry (CM2B4) was evaluated in all groups. CMCCs exhibited neuroendocrine areas associated with in situ or invasive SCC, overlying actinic keratosis or intratumoural squamous differentiation. When possible, cases were age, gender and site-matched across the different study groups.
Results: In the MCC group, MCV antigen was detected in 15/24 (63%) PMCCs, 0/13 (0%) CMCCs and 7/13 (54%) MCC metastases. Complete concordance (100%) of MCV-positivity was observed between 10 cases of primary MCC and associated metastatic MCC. All 70 non-MCC tumours, including those from both immunocompetent and immunosuppressed individuals, were negative for MCV protein expression.
Conclusions: The absence of MCV protein expression in the current series of CMCCs and non-MCC tumours concurs with earlier findings and suggests an MCV-independent oncogenic pathway for these tumours. Our systematic comparison of MCV expression in primary MCC and associated metastatic MCC revealed identical frequency and pattern of MCV-positivity. The frequency of MCV detection in the current series of PMCC is comparable to that previously reported using CM2B4 immunohistochemistry and falls within the range reported by molecular methods. Notably, the identification of MCV in only a proportion of MCCs in this study and many others invokes a role for additional etiologic factors in the pathogenesis of MCC.

501 Clinical Outcomes for a Large Cohort of Patients with Dermatofibrosarcoma Protuberans with Fibrosarcomatous Transformation.

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Background: Dermatofibrosarcoma protuberans (DFSP) is a locally aggressive cutaneous sarcoma characterized by storiform spindle cells. In some tumors, fibrosarcomatous transformation (DFSP-FS) occurs, typically with a sharply demarcated cellular area in a fascicular growth pattern. Several studies have shown DFSP-FS to variably increase risk of local recurrence and distant metastasis. We examined the clinical outcome in a large cohort of patients with DFSP-FS treated at a single institution.
Design: The pathology files were examined from 1985-2010 for patient cases with DFSP showing FS transformation. Clinical data and outcomes were tabulated.
Results: From 1985-2010, 776 patients with DFSP were identified with 9% (n=71) having FS transformation. The mean age of these DFSP-FS patients was 46 years (range: 18-79) with a male predominance (1.6:1). Ethnicity was known in 54 cases: Caucasian, 57%; Hispanic, 22%; Black, 15%; and Asian, 6% – percentages similar to our overall patient population. Common anatomic sites (n=71) included back (31%), head and neck (15%), abdomen (13%), chest (10%), upper extremity (10%), and groin (8%). The mean tumor size was 7.8 cm (range: 1.0-30.0). Where available (n=42), the median follow-up was 42 months (range: 1-377). Overall metastatic frequency was 21% (9/42); the median time to metastasis was 28 months (range: 18-361). The most common metastatic site was lung (78%, 7/9). Local recurrence was seen in 40% of DFSP-FS patients (17/42), and 41% of these recurrent cases with follow-up (7/17) developed metastatic disease. The median time to recurrence was 21 months (range: 4-309). Margins were microscopically positive in 48% of cases at resection (22/46); of these cases with follow-up, 47% (8/17) developed local recurrence, and 24% (4/17) developed metastatic disease. At last clinical follow-up, 62% of patients (26/42) had no evidence of disease, 21% (9/42) were alive with disease, 10% (4/42) died of disease, 5% (2/42) were alive with unknown disease status, and 2% (1/42) died of unknown cause. No patients with DFSP lacking FS transformation (n=705) were known to develop metastasis.
Conclusions: DFSP with FS transformation is an aggressive tumor with increased risk of local recurrence and metastasis. Only DFSP cases with FS change were found to metastasize in this large cohort, and the lung was the most common metastatic site. Close clinical follow-up for patients with DFSP-FS is warranted.

502 Postradiation Cutaneous Angiosarcoma after Treatment of Breast Carcinoma Is Characterized by *c-MYC* Amplification in Contrast to Atypical Vascular Lesions after Radiotherapy and Control Cases. Clinicopathologic, Immunohistochemical and Molecular Analysis of 61 Cases.

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Background: Postradiation cutaneous vascular lesions after treatment of breast carcinoma comprise a heterogeneous group of benign, atypical, and malignant lesions and are best regarded as points along a morphologic spectrum. There is a significant clinical, histological, and immunohistochemical overlap between atypical vascular lesions and cutaneous angiosarcomas and exact prognostication of single cases remains very problematic. We analysed a series of cutaneous angiosarcomas after treatment of breast cancer in comparison to control cases and cases of atypical vascular lesions with special emphasis of the expression and amplification of *c-MYC*.
Design: We examined cases from our routine and referral files and evaluated histologic, immunohistochemical and molecular features. The 61 cases were divided into control cases (4), cases in which a slight vascular proliferation was seen after radiotherapy of breast cancer (12), cases of atypical vascular lesions after radiotherapy (15), cases of postradiation cutaneous angiosarcomas (22), and cases of angiosarcomas of skin and soft tissues unrelated to radiotherapy (8).

Results: None of the control cases (2 M, 2 F, 20-76 years, hobnail hemangioma, hemangiolymphangioma, postirradiation colitis), of cases showing slight vascular proliferation, dermal fibrosis and inflammation after radiotherapy of breast cancer (12 F, 48-79 years), of cases of atypical vascular lesions after radiotherapy including benign lymphangiomatous papules and atypical vascular proliferations (15 F, 29-81 years), and of cases of angiosarcomas of skin and soft tissues unrelated to radiotherapy (3 M, 5 F, 25-92 years) showed an amplification of *c-MYC* by FISH-analysis. In striking contrast, in all cases of postradiation cutaneous angiosarcomas (22 F, 46-95 years) a *c-MYC* amplification was found by FISH-analysis in a variable number of counted nuclei. Immunohistochemically, a strong positive nuclear staining for c-myc and prox-1 was seen in cases of postradiation cutaneous angiosarcoma, whereas control cases, and cases of atypical vascular proliferation after radiotherapy were negative for c-myc, and stained only focally positive for prox-1.
Conclusions: The presence of *c-MYC* amplification represents an important additional diagnostic tool in the distinction of postradiation cutaneous angiosarcomas from atypical vascular lesions after radiotherapy.

503 Validation of Tandem Mass Spectrometry Study Using Laser Micro-Dissected Melanoma and Nevus Cells: Increase of Silver and Fatty Acid Synthase Protein Level in Melanoma.

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Background: The incidence of melanoma continues to rise every year, yet the ability to treat advanced disease has not changed. Previous studies from our group and others have recognized that proteins involved in tumorigenesis can be identified by mass spectrometry on formalin fixed paraffin embedded (FFPE) sections. Our study aims to identify melanoma markers on FFPE sections by comparing metastatic melanoma and benign nevus with improved protein extraction, gel separation and mass spectrometry analyses. We decided to study some of these significant proteins and determine if the mass spectrometric data can be validated and if this method can yield other novel biomarkers. Once this method is validated, additional proteins identified as significant using mass spectrometry can be analyzed on a large scale basis.
Design: Melanoma cells or melanocytes were harvested from laser micro-dissection and proteins were extracted and analyzed by Thermo-LTQ-XL mass spectrometer coupled to an Eksigent nanoLC-2D. Approximately 300 proteins were differentiately expressed between metastatic melanoma and nevi with high significance. We found two published possible melanoma markers, SILV and fatty acid synthase (FAS), to validate the mass spectrometry results using immunohistochemical stains. FFPE blocks were retrieved from our archives as follows: 12 benign nevi, 7 dysplastic nevi, and 13 melanoma. Stain patterns were scored based on extent (0-3) and intensity (0-3). The product of the extent and intensity was determined generating a score of 0-9 for each. These were then divided as follows: 0-1=0; 2-4=1+; 5-6=2+; 7-9=3+.
Results: Higher immunoreactivity for SILV was detected in the melanoma cases than in the benign and dysplastic nevi cases which was statistically significant ($p<0.001$). 8 out of the 13 melanoma cases had a score of 3+, in contrast 19 out of 20 benign or dysplastic nevi cases expressed minimal or absent immunoreactivity (< or = 1+). Similar findings were found for FAS. 8 of 9 melanoma cases had high expression (2+ or 3+) whereas only 2 of 14 benign nevus and dysplastic nevus had high expression ($p < 0.0015$).
Conclusions: This study showed that laser micro-dissected FFPE sections coupled with mass spectrometry can identify biomarkers when comparing metastatic melanoma with a benign nevus. Immunostains for two markers validated the mass spec findings. We will continue to examine proteins that were found to be significant by mass spectrometry in metastatic melanoma in a larger scale future study.

504 Utility of p53 Immunostaining in Inflamed Proliferative Skin Lesions.

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Background: Immunohistochemical (IHC) staining for the p53 tumor suppressor protein has been used as an ancillary tool in distinguishing benign from malignant epithelial lesions. Literature has shown that the majority of malignant lesions show strong and diffuse p53 immunoreactivity while benign proliferative skin lesions typically show a basal/parabasal immunoreactivity. Inflamed seborrheic keratoses with reactive atypia present a diagnostic challenge as these benign lesions may have overlapping histologic features with well-differentiated squamous carcinomas. This study evaluates the utility of p53 IHC in a variety of benign and malignant proliferative skin lesions.
Design: Thirty cases were identified for study and included cases diagnosed as invasive squamous cell carcinoma (SCC) (n = 3), SCC in-situ (n = 13), inflamed seborrheic keratosis (SK) (n = 7), inflamed verruca vulgaris (n = 3), and non-inflamed verruca vulgaris (n = 4). Of note, four of the SCC in-situ lesions were arising within a SK. Immunohistochemical staining for p53 (1:20 dilution; Ventana, Tucson, AZ) was performed on all cases and the pattern of nuclear staining within the keratinocytes was recorded as either basal/parabasal or diffuse.
Results: The majority of invasive SCC (66%; 2/3) and SCC in-situ (85%; 11/13) demonstrated diffuse positivity for p53. One of the SCC in-situ lesions arising within an SK demonstrated only rare basal cell positivity. The majority of SK's (71%; 5/6) showed basal/parabasal staining; while unexpectedly a subset (29%; 2/7) of the inflamed SK's showed diffuse staining. All of the verruca vulgaris lesions, inflamed or non-inflamed, stained in a basal cell-only pattern for p53.
Conclusions: In concordance with previous literature, the majority of malignant lesions studied (invasive SCC and SCC in-situ) demonstrated a pattern of strong, diffuse positivity for p53. The basal cell-only staining of all the verruca vulgaris lesions is also in concert with previous studies. Interestingly, a minority of inflamed SK's showed a strong and diffuse pattern of p53 immunoreactivity while one SCC in-situ lesion arising within a SK had only rare basal cell positivity. These findings suggest a limitation to the utility of p53 in distinguishing benign from malignant lesions. The diffuse positivity within an inflamed SK raises the possibility that a subset may represent pre-malignant lesions.

505 Loss of the *PRKAR1A* Gene Locus Identified by Conventional Cytogenetic and Fluorescence In Situ Hybridization Analysis in Solitary Superficial Angiomyxoma.

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Background: Superficial angiomyxoma is a distinct but often poorly recognized benign cutaneous neoplasm. Superficial angiomyxoma most frequently arises sporadically, but it may also occur as a feature of the autosomal dominant disorder Carney complex (CNC). *PRKAR1A* is a tumor suppressor gene on chromosome 17 that is mutated in some Carney complex (CNC) patients. Superficial angiomyxoma has not previously been subjected to cytogenetic or molecular cytogenetic analysis.
Design: Conventional cytogenetic analysis was performed on a representative portion of a solitary superficial angiomyxoma arising on the thigh of a 54 year old male. In addition, a 192 kb bacterial artificial chromosome (BAC RP11-120M18) clone identified as spanning the *PRKAR1A* (17q24) locus was fluorescently labeled to conduct higher resolution analysis. After establishing the specificity of the probe and coupling it with a CEP 17 copy number control probe, dual color FISH studies were performed to assess the *PRKAR1A* copy number on destained metaphase cells and FFPE tissue sections of the cytogenetically analyzed superficial angiomyxoma as well as FFPE tissue sections on one additional superficial angiomyxoma, two cases of cutaneous focal mucinosis and one negative control.
Results: The following abnormal chromosomal complement was detected in the 54 year old male's superficial angiomyxoma: 46,XY,ins(17;17)(q25;q12q21). FISH studies revealed homozygous loss of the *PRKAR1A* locus in the cytogenetically analyzed superficial angiomyxoma. The remaining cases were negative for *PRKAR1A* loss by FISH. A mutational mechanism other than deletion such as a submicroscopic mutation resulting in a truncated or inactive protein may be responsible for *PRKAR1A* inactivation in the single case of FISH negative sporadic superficial angiomyxoma.
Conclusions: The role of germline *PRKAR1A* mutations in CNC, to include the presence of superficial angiomyxomas, is well recognized. The data from the current study showing homozygous loss of the *PRKAR1A* locus in one non-CNC superficial angiomyxoma supports a somatic role of the *PRKAR1A* tumor suppressor gene in sporadic superficial angiomyxoma as well.

506 Utility of SOX10 as a Reliable Marker for Melanocytes in Sun-Damaged Skin.

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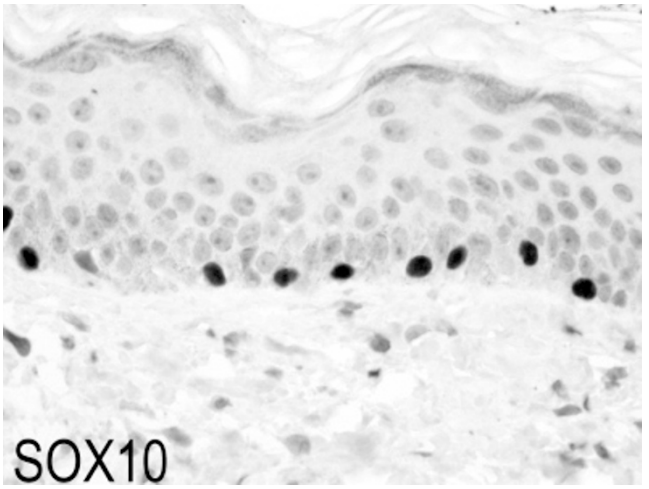
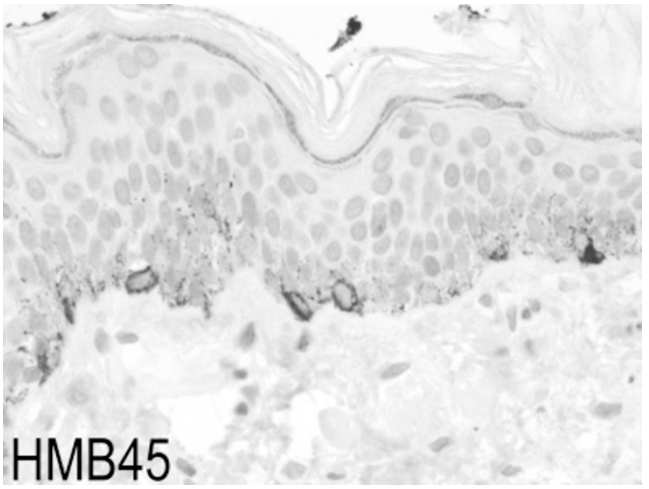
Background: In sun-damaged skin, it is sometime problematic distinguishing between atrophic actinic keratosis and atypical melanocytic proliferations on routine Hematoxylin and Eosin stain, especially when there is pseudomelanocytic proliferation. Attempts to resolve this diagnostic challenge with Melan A Immunohistochemistry has been reported to result in spurious staining results and diagnostic errors with dire consequences. This study examines the usefulness of a relatively new melanocytic marker (SOX10) in such cases.
Design: 23 consecutive cases of atrophic actinic keratosis with features of lentigo maligna-like features were stained with antibodies against HMB45 (a well established cytoplasmic melanocytic marker) and SOX 10 (a new marker located in the nucleus). Immunohistochemistry was performed using modified avidin-biotin detection method with antigen retrieval. For each antigen, the immunostained slides were evaluated for location of positive staining reaction – being it in melanocytes and/or in keratinocytes.
Results: In all 23 cases, melanocytes in the basal layer of the epidermis stained positive with both HMB45 and SOX 10 in the cytoplasm and nuclei respectively. Although the number and distribution of cells stained by both antibodies were comparable, the nuclear staining reaction of SOX10 is more readily discerned and more easily interpreted compared with HMB45's cytoplasmic staining reaction and associated non specific granular staining reactions noted in keratinocytes. No positive staining for SOX10 was detected in the dysplastic keratinocytes (Actinic Keratosis cells) in any of the 23 cases.





Conclusions: Immunohistochemical stain for SOX10 (a nuclear stain) is a reliable marker for distinguishing between melanocytes and keratinocytes in sun damaged skin; and does not produce the false positive (cytoplasmic) staining reaction reported with Melan A in such situations.

507 Inflammatory Morphea with T Cell Receptor Gamma Gene Clonality.

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Background: Inflammatory morphea has been described as a histologic as well as clinical simulant of interstitial mycosis fungoides (MF). Specifically, in early inflammatory morphea, inflammation is in a prominently interstitial pattern with minimal sclerosis, such that significant overlaps with interstitial MF may exist. In clinical practice, we encountered two cases of inflammatory morphea which demonstrated not only histological and clinical similarities with interstitial MF but also T-cell receptor gamma (TCRg) gene clonality by gene rearrangement (GR) analysis. With time, both cases evolved into clear-cut morphea. Here, we performed retrospective TCRg GR studies on more cases of morphea to assess GR analysis as a potential pitfall.

Design: A total of 21 cases of morphea were identified through our pathology information system, and diagnoses were confirmed by microscopic review and correlation with clinical information. Formalin fixed paraffin embedded tissue blocks from these cases were submitted to TCRg GR analysis by polymerase chain reaction (PCR) and interpreted in standard fashion.

Results: Patients ranged from 25 to 77 years of age (median 46 years), and were predominantly females (2:1). The majority of specimens came from truncal locations, such as breast, chest, abdomen and back. Based on the degree of sclerosis, these 21 cases were divided into two groups: 9 cases of group A (minimal sclerosis with prominent interstitial infiltrate) and 12 cases of group B (well-developed sclerosis with variable perivascular or nodular infiltrate). The amount of interstitial dermal inflammation appeared inversely related to the degree of sclerosis. Four cases were positive for clonal TCRg gene by PCR. The remaining 17 cases were negative for clonality. The positive cases involved 3 females and 1 male, ranging in ages from 41 to 61 years old. The specimens were taken from abdomen (2), breast (1) and upper chest (1). Histologically, Three cases were classified as group A and 1 as group B.

Conclusions: A small subset of inflammatory morphea cases may show TCRg gene clonality. Therefore, the distinction between early inflammatory morphea and interstitial MF should not be exclusively based on the result of TCRg GR study result. Definitive distinction of inflammatory morphea from interstitial MF will depend upon clinical as well as pathologic evolution of the disease over time with the findings of more classic lesions.

508 Misregulation of RAD50 in Melanoma Cells.

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Background: Ultraviolet radiation has been linked to increased risk of skin cancer including melanoma. One hypothesis is the accumulation of damaged DNA by way of UV radiation, leading to DNA double stranded breaks (DSB). When this reaches an irreparable sum the cell activates the pathway of cell death or apoptosis. Previously, we determined that DNA DSBs, as detected by histone H2AX phosphorylation, are increased in human melanoma tissue. We decided to determine if the downstream effectors of DNA DSB, namely RAD50 expression are altered in human melanoma cells.

Design: Eleven melanoma, 7 severely and 11 mildly dysplastic melanocytic nevi were retrieved from our archives. Age, sex, and location were not significantly different between the melanocytic lesions ($p > 0.5$). Routine immunohistochemistry was performed with commercially available antibodies against RAD50 (Cell Signaling). The intensity of staining was scored from nil to high (0-3). The percentage of cell staining was scored as 0 (none), 1 (<25% staining), 2 (<50% staining), and 3 (>50% staining). The intensity was multiplied by the percentage and the product was categorized as follows: 0-1 is 0; 2-3 is 1+; 4-5 is 2+; 6-9 is 3+. Chi square analysis was used to determine statistical significance with a $p < 0.05$ considered significant.

Results: RAD50 staining is significantly increased in melanoma (82%) cases compared to cases of mildly (27%) and severely (29%) dysplastic melanocytic nevi. Severely and mildly dysplastic melanocytic nevi had average staining intensities of 1.25 and 1, respectively. The majority of the melanoma cases demonstrate intense 3+ staining with an average staining intensity of 2.45. Both staining intensity and percentage of positive cells were statistically significant ($p<0.001$). Interestingly, RAD50 localized to cytoplasm in 7 of 9 (78%) positive melanoma cases, whereas all (100%) of the RAD50 staining localized to the nucleus in severely and mildly dysplastic melanocytic nevi ($p < 0.01$).

Conclusions: This is the first study that demonstrates activation and misregulation of the DNA repair pathway in human melanoma cells. Previous studies have shown increased histone H2AX phosphorylation in melanoma, suggesting an increase in DNA DSB's. In this study, the staining features of RAD50, a component of an essential DNA DSB repair protein, are clearly increased in melanoma cells. Interestingly, the majority of RAD50 staining in melanoma cells is cytoplasmic indicating aberrant localization of at least one DNA DSB repair protein. Future studies will determine the mechanism of aberrant RAD50 localization in human melanoma cells.

509 KIT in Atypical Acral Nevi – An Immunohistochemical and Genetic Appraisal.

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Background: Mutations in KIT, present in up to 23% of acral melanomas, are clinically significant because of the availability of KIT-targeted therapies. A recent study indicates a correlation between KIT mutations and immunohistochemical expression of CKIT in acral melanoma. Given this, our aim was to confirm the utility of CKIT expression as a screening tool for KIT genotyping in atypical acral nevi – putative precursor lesions of melanoma. An additional aim was to ascertain the frequency of KIT mutations in atypical acral nevi.

Design: Immunohistochemical staining for CKIT was performed using a polyclonal rabbit anti-human C-KIT antibody. Staining criteria were the following: negative= staining in <10% of cells, 1= staining in 11-49% and 2= staining >50% of cells. Intensity was graded as follows: negative =0, weak staining = 1, moderate staining = 2 and strong staining = 3. Genomic amplification *via* PCR and direct sequencing were performed on KIT exons commonly mutated in acral melanomas such as 11, 13, and 17, from formalin-fixed paraffin-embedded tissue samples of atypical acral nevi (23) ranging in severity from mild (9), moderate (10), and severe (4). The control group included acral nevi without atypia (19) and acral melanoma (10). For purposes of statistical analyses: cases with 11% or more staining of cells were compared with negative cases and cases with a staining intensity of 1 or higher were compared with the negatives.

Results: Immunohistochemical analyses of acral nevi revealed the following: positive staining (11% or more) with an intensity 1 or more was noted in 18/22 (82%) of cases with atypia (5 with mild; 9 with moderate and 4 with severe atypia) and in 13/17 (76%) of cases without atypia. No statistically significant differences were noted between acral nevi with and without atypia in proportion and intensity of staining. Genomic analyses of exon regions revealed no abnormalities in "hotspots" frequently associated with point mutations in acral melanomas in acral nevi with and without atypia.

Conclusions: Our findings argue against the utility of immunohistochemical expression of CKIT as a method of prioritizing cases for KIT genotyping. Atypical acral nevi do not exhibit genetic alterations in "hotspots" frequently mutated in acral melanomas.

510 Levamisole-Induced Vasculopathy and Associated Skin Necrosis in Crack/Cocaine Users.

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Background: Levamisole had been used as an anti-helminthic as well as an immunomodulatory drug before being taken off the market in 2003. In therapeutic settings, levamisole use has been linked to agranulocytosis and skin necrosis. Starting

in 2004, this drug has been reported in a high proportion of seized cocaine in North America and Europe. Cases of febrile neutropenia were observed in users of crack/cocaine contaminated with levamisole. Cases of skin necrosis in this setting have recently been reported in lay literature. Levamisole induced skin necrosis, when observed in therapeutic setting, improves gradually after discontinuation of medication and in some cases, immunosuppression.

Design: Three patients presented to St. Michael's Hospital with history and/or laboratory evidence of crack/cocaine usage and development of skin lesions.

Results: All three patients were young Caucasian females who presented over a period of three months with rapidly progressive painful necrotic skin lesions. The lesions were predominantly located on cheeks, ears, breasts, and buttocks. Extremities were involved in two patients and upper respiratory tract mucosa in one. Skin biopsy in all cases revealed thrombotic vasculopathy with foci of vasculitis in a non-specific pattern, with overlying ischemic ulceration of skin and soft tissues. The first patient was given a provisional diagnosis of cryoglobulinemic vasculitis and treated with plasmapheresis, which resulted in rapid improvement in her skin lesions. Two relapses were successfully treated with plasmapheresis and steroids. Considering the first patient's prompt response to plasmapheresis, the subsequent two patients were offered plasmapheresis as well and had improvement or stabilization of the skin lesions.

Conclusions: Levamisole is frequently added to crack/cocaine to augment the effect; we report three female patient who developed vascular thrombosis and skin necrosis after using levamisole-contaminated crack/cocaine.

511 Proliferative Nodule Arising within Congenital Melanocytic Nevus: Histologic, Immunohistochemical and Molecular Analyses of 43 Cases.

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Background: The histopathologic interpretation of proliferative nodules (PN) in congenital melanocytic nevi (CMN) can present significant challenges since some PN may exhibit atypical features which make the histologic distinction from melanoma difficult.

Design: We compared histologic features, Ki-67%, PHH3 (number of positive cells per 10 high power fields), and CD117% expression by immunohistochemistry (IHC) in 18 benign and 25 atypical PN (from 41 patients) to that of background CMN (of these 43 cases), 10 CMN and 3 dermal melanomas (MM). In addition, we evaluated the presence of NRAS, BRAF, KRAS, HRAS, and GNAQ mutations in all groups using the SNaPshot® Multiplex System.

Results: Follow-up was available on 19 patients (9 benign PN, 10 atypical PN) (range: 2 to 19 years; median: 8 years) and all were alive with no evidence of disease. Specific histologic features of atypical PN: sharp demarcation, expansile growth, epidermal effacement, pleomorphism, and increased mitoses differed significantly from benign PN using Fisher's exact test. Comparison of the IHC expression in the 5 groups was performed by analysis of variance, with Holm-Sidak post-hoc test. Immunohistochemical results (Table 1) showed that Ki-67% and PHH3 scores, but not CD117% expression, were statistically different ($P < 0.05$) for benign PN vs. atypical PN and MM; but not atypical PN vs. MM. Molecular analysis (Table 1), revealed no significant difference in mutation frequency although BRAF and NRAS mutations were common.

Table 1: Summary of immunohistochemical and molecular findings

		CMN	Background CMN	Benign PN	Atypical PN	MM
Ki-67%	Mean±SD	0.2±0.03 (n=10)	0.09±0.03 (n=37)	1.01±0.28 (n=13)	7.23±1.45 (n=22)	5.87±3.3 (n=3)
PHH3	Median (range 25-75%)			1 (1-1.25) (n=18)	5 (2-8) (n=25)	10 (6.25-11.5) (n=3)
BRAF	Positive mutation/ Number of samples	1/5	10/35	4/16	7/19	0/3
NRAS	Positive mutation/ Number of samples	-	13/27	5/11	6/14	1/3

Conclusions: The data suggest that histologic features, Ki-67 and PHH3 expression are the strongest parameters to distinguish between benign PN vs. atypical PN and MM. No criteria could distinguish between atypical PN and MM. This suggests that atypical PN are distinct borderline lesions between benign PN and MM. Although numerous mutations are detected in the samples, the diagnostic utility of molecular analysis in this regard is limited.

512 Podoplanin (D2-40) Expression in Cutaneous Fibrohistiocytic Tumors: A Clinicopathologic and Immunohistochemical Study of 43 Cases.

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Background: Podoplanin (D2-40) is widely used as a marker for lymphatic endothelial cells; however, it has also been found to be expressed in a number of non-vascular tumors. In normal human skin, podoplanin expression is confined to the basal cell layer of the epidermis, basal layer of sebaceous glands, and the basal layer of the outer root sheath of hair follicles; thus, expressed in many primary skin neoplasms. Lately, podoplanin expression has been found in some cutaneous fibrohistiocytic neoplasms but there is limited and conflicting data on this regard. We sought to analyze the expression of podoplanin in 43 cases of cutaneous fibrohistiocytic tumors including conventional and cellular dermatofibromas, to assess its potential diagnostic utility.

Design: A total of 43 fibrohistiocytic tumors form the basis of our study, including 16 cases of conventional dermatofibromas and 27 cases of cellular dermatofibromas. Available histologic material was reviewed, and 43 cases with unequivocal histologic features of either tumor were included in this study. Immunohistochemical studies were performed in selected formalin-fixed, paraffin-embedded blocks in all cases. The antibody employed was D2-40 (podoplanin) protein (1:200 dilution, DAKO, Carpinteria California). Appropriate positive and negative controls were run concurrently for the marker tested. Immunolabeling for podoplanin was scored as negative, focally positive or diffusely positive cytoplasmic staining. We defined negative as no reactivity, focally positive as staining in 1% to 25% of tumor cells and greater than 25% was defined as diffuse staining.

Results: Podoplanin immunoexpression was present in 15/27 cases of cellular dermatofibromas; 6 cases were diffusely positive (22.2%), 9 cases were focally positive (33.3%), and 12 cases were negative (44.4%). Podoplanin immunoexpression was present in 5/16 cases of conventional dermatofibromas; 1 case was diffusely positive (6.25%), 4 cases were focally positive (25%), and 11 cases were negative (68.75%).

Conclusions: In summary, the data generated in this study differs from other studies and suggest that podoplanin does not represent a sensitive marker in cutaneous fibrohistiocytic tumors. These results also suggest that podoplanin is more commonly expressed in cellular fibrohistiocytic lesions and thus may be used as a complementary stain to CD34, due to the difficult distinction between dermatofibrosarcoma protuberans and cellular dermatofibromas, especially in superficial biopsies.

513 Immunohistochemical Expression of GSK-3β and CDC25A in Cutaneous Lymphomas.

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Background: Tyrosine phosphatase Cdc25A is required for G1 to S progression in cell cycle. GSK-3β, a serine/threonine kinase, regulates cell cycle by phosphorylating key proteins and targets Cdc25A for proteolysis. GSK-3β inactivation correlated strongly with Cdc25A accumulation in human tumors. Inhibitors of both enzymes are attractive targets for anticancer therapy development. Here we evaluated the expression of Cdc25A and p-GSK-3β (phosphorylated inactivated form) in cutaneous lymphomas.

Design: Tissue microarray (TMA) was created using material obtained at our Pathology Department. Diagnoses included systemic and cutaneous anaplastic large cell lymphoma (ALCL), lymphomatoid papulosis (LyP), mycosis fungoides (MF), MF with large cell transformation (MF-LCT), primary cutaneous B-cell lymphomas (BL), systemic BL with secondary cutaneous involvement, and cutaneous lymphoid hyperplasia (LH). Cdc25A and p-GSK-3β immunohistochemical staining of TMA was evaluated for immunoreactive lesional cells and scored negative/low (<20% of positive cells) or high (≥20% of positive cells).

Results: P-GSK-3β immunoreactivity was cytoplasmic and strong in all lymphoma types (Table). Staining for Cdc25A was mostly cytoplasmic with some perinuclear accentuation. No or low levels of Cdc25A were observed in most cutaneous ALCLs, LyPs, cutaneous and systemic BLs with secondary cutaneous involvement. In contrast, high levels of Cdc25A were noted in some MFs and aggressive lymphomas, such as systemic ALCL, MF-LCT, and a case of primary cutaneous diffuse large B-cell lymphoma of leg type. Interestingly, p-GSK-3β was overexpressed in cutaneous LH, while Cdc25A was negative or low.

GSK-3β and Cdc25A expression in lymphoma TMA

	GSK3β		Cdc25A	
	neg/low	high	neg/low	high
systemic ALCL	25%	75%	50%	50%
cutaneous ALCL	14%	86%	86%	14%
LyP	14%	86%	86%	14%
MF	0%	100%	44%	56%
MF-LCT	11%	89%	33%	67%
cutaneous BL	0%	100%	82%	18%
systemic BL	0%	100%	100%	0%
cutaneous LH	0%	100%	100%	0%

Conclusions: The differential expression of Cdc25A suggests that p-GSK-3β and Cdc25A axis may play a role in some aggressive forms of cutaneous lymphomas. The discordant expression of Cdc25 and p-GSK-3β indicates that non-Cdc25A cell cycle pathways may be involved in indolent skin lymphomas such as cutaneous ALCL. Our findings may be useful for directing future specific inhibitor therapy, however, larger studies are necessary to establish the role of p-GSK-3β and Cdc25A in these lesions.

514 Expression of the Stem Cell Marker CD133 (Prominin-1) in Eccrine and Apocrine Skin Tumors.

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Background: Human CD133 (human prominin-1) is expressed by various stem and progenitor cells, and has been detected in a small population of neoplastic cells. We describe the CD133 immunohistochemical expression of eccrine and appocrine tumors of the skin.

Design: For immunohistochermical study, a retrospective search retrieved 38 previously diagnosed skin eccrine and apocrine adnexal tumors from the files of the Department of Pathology of the University Hospital Complex of Albacete. All cases were reviewed, and adnexal skin tumors were diagnosed according to the criteria of the WHO classification, including: Eccrine spiradenoma (n=3), Nodular hidradenoma (n=5), Eccrine hidrocystoma (n=3), Poroma (n=5), Porocarcinoma (n=1), Syringocystoadenoma papilliferum (n=3), Chondroid syringoma (n=4), Syringoma (n=6), Cilindroma (n=2), Hidradenoma papilliferum (n=2), apocrine hidrocystoma (n=3) and apocrine carcinoma (n=1). Other types of skin epithelial tumors (5 basal cell carcinomas, and 5 squamous cell carcinomas) were also included in this study to compare the results obtained with those obtained in sweat gland tumors. Slides were treated with heat-induced epitope retrieval and immunostained with three different anti CD133 monoclonal antibodies: the AC133 monoclonal antibody (Miltenyi Biotec, Germany), the W6B3C1 o 292C3 monoclonal

antibody (Miltenyi Biotec, Germany), and the AC141 monoclonal antibody (Miltenyi Biotec, Germany). CD133 detection was performed by using the EnVision system-HRP (Dako, Glostrup, Denmark) in the DakoCytomation Autostainer platform.
Results: CD133 was not expressed in any of the basal cell carcinomas and squamous cell carcinomas tested. The overall immunohstochemical staining pattern observed in the studied adnexal tumors was closely related to tissue architecture, being positive in the ductal or acinar areas of the eccrine tumors. CD133 immunoreactivity was mainly seen in the apical/endoluminal surface of duct-like structures or cysts of all the benign and malignant eccrine tumors.
Conclusions: Our findings demonstrate that CD133 protein is not restricted to stem cells. CD133 is widely expressed on differentiated luminal and ductal epithelial cells of different eccrine skin adnexal tumors.

515 Comparative Analysis of EGFR Immunohistochemical Expression and Mutational Analysis in Metastatic Basal Cell Carcinomas.

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Background: Basal cell carcinoma is a common and indolent tumor that rarely metastasizes (incidence: 0.0028 to 0.5%). The median survival of metastatic basal cell carcinoma (MBCC) is 10-14 months and the 5-year survival is approximately 10%. One of our prior studies examined EGFR immunohistochemical expression in MBCC, which showed positive expression in all examined cases. A literature search did not reveal any studies on EGFR mutation analysis in MBCC. Given the poor prognosis and desire of oncologists to try newer targeted therapy as a last resort, such as Imatinib, examination for EGFR mutations in these aggressive tumors could be potentially fruitful.
Design: Seven samples were selected of metastatic disease to the following: kidney(1), lung(2), lymph node(2), soft tissue(1), and vetebra (1). The vetebral metastasis was excluded from mutation analysis due to decalcificied nature of specimen. Tumors were stained for EGFR (DakoCytomation pharmDx kit) as directed. RNA was extracted from corresponding paraffin blocks using the RNAeasy FFPE kit, and DNA was extracted using the QIAamp DNA mini kit. In-house real time PCR was used to identify variant III EGFR. The QIAGEN EGFR PCR kit was used to identify 28 of the most common mutations of the EGFR gene.
Results: There was positive EGFR immunohistochemical expression in all seven cases. No EGFR mutations were detected in the 4 samples that could be analyzed (wild type). Mutation analysis for EGFR variant III showed similar absence of the mutation in 4 samples. Three cases had insufficient tissue for complete mutational analysis.

Mutation Analysis

Sample	Metastatic Site	EGFR IHC	EGFR vIII mutation	EGFR Mutation
1	Kidney	2+	Absent	Absent
2	Lung	2+	Absent	Absent
3	Lymph Node	2+	Absent	Absent
4	Lymph Node	2+	QNS	Absent
5	Lung	2+	Absent	QNS
6	Soft tissue	3+	QNS	QNS
7	Vetebrae	2+	n/a	n/a

IHC: Immunohistochemistry, scored 1-3+; QNS: Quantity not sufficient for analysis

Conclusions: This case series of MBCC did not reveal mutations within EGFR genes. Mutational analysis does not correlate with EGFR IHC expression, and positive IHC expression can be misleading and lead to inappropriate treatment as recent studies have shown that patients with EGFR mutations are the most likely subset to respond to selective anti-EGFR tyrosine kinase inhibitors. Recent studies suggest a new direction, targeting mutations in the hedgehog signaling pathway and associated targeted therapy.

516 Expression of EZH2 and ALDH1 in Metastatic Malignant Melanoma Affects Survival.

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Background: Aldehyde dehydrogenase 1 (ALDH1) and enhancer of zeste homolog 2 (EZH2) are two markers important in normal progenitor stem cells. ALDH1 is a detoxifying enzyme that may have a role in the early differentiation of stem cells, through its role in oxidizing retinol to retinoic acid. A polycomb group protein, EZH2 regulates the G2 to mitosis transition and increased cell proliferation. ALDH1 and EZH2 have been linked to the progression of many human cancers, specifically breast and prostate cancer. To date, there is no study addressing the clinical significance of ALDH1 and EZH2 expression in primary versus metastatic malignant melanoma (MM).
Design: Our study consisted of 31 cases of MM: 12 with lymph nodes metastasis and short-term survival, 8 with lymph node metastases and long-term survival and 11 primary MMs. All cases were stained for ALDH1 (BD Biosciences)and EZH2 (BD Biosciences). EZH2 expression in the melanocytes was quantified by automated image analysis (Aperio) using a 3 point scale, 0=no staining, 1=weak (<25% cells staining), 2=moderate (25-75% cells staining) and 3=strong (>75% cells staining). Scores of 0 and 1 were considered low expression and 2 and 3 high expression. For ADH1 staining intensity (weak, strong) and precentage of staining melanocytes were evaluated. Correlation between marker expression and survival was evaluated using the Log Rank test.
Results: Primary MMs with high expression of EZH2 had shorter survival than those with low expression (p=0.0330). The median survival length for primary MMs with high EZH2 expression was 317 days as compared to 2262 days for those with low EZH2. The median survival length for patients with lymph node metastases was 201 days for those with high EZH2 and 4288 for those with low EXH2 expression (p<0.0001). ALDH1 expression was not associated with a difference in survival.
Conclusions: High expression of EZH2 in primary malignant melanomas and lymph node metastases correlates with poor survival. Our study opens the field to further investigate EZH2 utility as a biomarker of poor prognosis in MM.

517 Expression of p63 Protein and Its Variants Switches MCPyV-Positive Merkel Cell Carcinoma towards a More Aggressive Tumor.

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Background: Prediction of clinical evolution in Merkel cell carcinoma (MCC), a rare and potentially aggressive tumor, is notoriously uncertain. In our previous experience, p63 expression in MCC represented an independent predictor of poor prognosis. In addition, recently it has been suggested that the presence of Merkel cell polyomavirus (MCPyV) is an indicator of adverse prognosis. Thus, to investigate on significance of the co-expression of p63 and MCPyV, we examined a series of MCC from different Institutions.
Design: We collected data on 72 MCCs; 27 cases were tested for p63 expression by immunohistochemistry (IHC) alone, while the remaining 45 were studied for p63 status by IHC, RT-PCR and FISH and for the presence of MCPyV by PCR.
Results: p63 expression, detected in 52.7% of cases by IHC, was associated with decreased OS (p=0.041) and DFS (p<0.001). In the 45 MCCs investigated with both IHC and RT-PCR a variable expression pattern of the different p63 isoforms was found in 25 out of 26 IHC-positive MCCs. In these same lesions at least one of the N terminal p63 isoforms was detected in the primary MCC. Accordingly, TAp63α was the most frequently expressed isoform. No p63 isoform was found in the 19 cases negative for p63 at IHC. P63 gene amplification was observed in only one case. Clonal integration of MCPyV DNA sequences was evidenced in 84.4% of MCCs. Expression of p63 protein in MCPyV-positive MCC cases was associated with an increased risk of death (p=.001) whereas p63 negativity indicated a less aggressive behaviour.
Conclusions: The present data indicate that clonal integration of MCPyV DNA sequences was not per se related to prognosis in our series while the expression of p63 protein and its variants in MCPyV-positive MCC cases related to a significant risk of death of disease, higher that staging or any other factor.

518 Melanoma of Unknown Primary Origin: Decreased Survival for Stage 4 Patients.

EC Rushing, R Tuthill. The Cleveland Clinic Foundation, OH.
Background: There is no consensus within the current literature on the prognostic significance of metastatic melanoma of unknown primary origin. Several studies have shown no survival advantage for patients with metastatic melanomas of unknown origin, and others have shown improved outcomes for these patients. We performed a retrospective study to determine if patients with metastatic melanoma of unknown primary origin have increased survival when compared to those with a known primary melanoma of the same stage.
Design: We conducted a retrospective analysis of patients within our institutional anatomic pathology database, with a pathologic diagnosis of "metastatic melanoma" between 1999 and 2008. 238 cases met the inclusion criteria; 199 of those had metastatic melanoma of known primary origin and 39 had metastatic melanoma of unknown primary origin. Patients were classified by the AJCC staging system (2002). The two groups were compared using the two sample T-test for continuous clinical measures, the Pearson's Chi-square or Fisher's exact test for categorical measures, and Kaplan-Meier curves with log- rank analysis for overall survival.
Results: There was statistically significant decreased survival for stage 4 melanoma patients of unknown primary origin compared to those of known primary origin (p=<.001). Stage 4 patients of known primary origin had a median survival of 20.0 months (95% CI: 14.0, 27.0) compared to those in the unknown primary group with a median survival of only 8.0 months (95% CI: 5.0, 10.0). No significant survival difference was found between the two groups for those with initial metastasis to lymph nodes only (stage 3 disease). Our study also confirmed a significant increase in survival for all patients with stage 3 disease (median survival of 69.0 months, 95% CI; 45.0, 110.0) when compared to patients with stage 4 disease (median survival of 14.0 months, 95% CI: 10.0, 20.0; p=<0.001).
Conclusions: Our study found that patients with melanoma of unknown origin had a statistically significant decreased survival for stage 4 disease when compared to those of the same stage with a melanoma of known origin. Between the known and unknown origin groups, there was no difference in survival for those that presented with initial metastatic disease to lymph nodes (stage 3 disease). All of the melanoma patients with stage 3 disease survived significantly longer than those with metastases to other organs. These findings and the mixed results of other studies suggest that more research is needed to better understand the complex nature of stage 4 melanomas of unknown primary origin.

519 Transplant Melanoma: An Emerging Recipient Risk.

EC Rushing, V Krishnamurthi, C Farver, R Tuthill. The Cleveland Clinic Foundation, OH.
Background: Aggressive donor transmitted melanoma is an emerging recipient risk. Malignant melanoma is the most common malignancy transmitted to transplant recipients comprising an estimated 28% of allogenic tumors. Any patients with a known history of melanoma are excluded from donation, so all donors that transmit malignancy have no prior history of disease. Here we report on 5 patients, all organ recipients, who acquired a highly aggressive melanoma from a 32 year old female donor.
Design: The authors conducted a retrospective search of patients, within our institutional anatomic pathology database, with a pathologic diagnosis of "metastatic melanoma" of

unknown primary origin between 1999 and 2008. Three cases of metastatic melanoma of unknown primary origin diagnosed after organ transplant were identified and prompted further chart review, histopathologic review of autopsy findings, review of donor registry information, and discussion with transplant and clinical care physicians.
Results: Five patients, all organ recipients, acquired widespread metastatic melanoma from one 32 year old female donor. A review of the donor chart showed that the donor died suddenly from an intracranial hemorrhage, had no known history of melanoma and did not have an autopsy to confirm her cause of death. The lung and heart transplant recipients both presented with respiratory distress and died of multi-system organ failure within two months of receiving their transplanted organs. Their cause of death, widespread aggressive metastatic melanoma, was identified by autopsy at our institution. PCR testing of autopsy tissue from the lung recipient showed the tumor to be derived from the donor organ. The remaining 3 organ recipients (pancreatic, kidney and liver) also presented within 3 months of their transplants with diffuse metastatic disease.
Conclusions: This report of 5 patients, all organ recipients, who acquired a previously undiagnosed, highly aggressive melanoma from a young multi-organ donor, presents some of the diagnostic, pathologic and clinical dilemmas associated with the emergent organ donation process.

520 Syringocystadenocarcinoma Papilliferum In Situ of the Penis Associated with Oncogenic HPV Infection.

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Background: Syringocystadenocarcinoma papilliferum (SCACP) is the malignant version of syringocystadenoma papilliferum (SCAP), which is a benign tumor of either eccrine, apocrine or apoeccrine origin that typically occurs on the scalp or face. SCACP is a rare phenomenon with only 10 cases reported, the vast majority arising from longstanding SCAP lesions. SCACP in situ (SCACP-IS) is an even rarer phenomenon with only 4 cases having been described. None of these have been reported in the genitalia (although one case occurred in perianal skin), and there has been no reported association with HPV infection.
Design: Histological examination, immunohistochemical characterization, and molecular HPV subtyping were performed on a routinely processed excisional biopsy. A clinical review was also performed.
Results: Histomorphology showed classic features for syringocystadenocarcinoma papilliferum which were confined to the surface epithelium with no evident breach of the basement membrane consistent with in situ disease. The lesion demonstrated malignant cytomorphology and there was no background evidence of a precursor lesion. Immunohistochemical analysis was also consistent with positive staining for Cam5.2, CK7, CEA and EMA. CK5/6, GCDFP, ER, AR and p63 were negative within lesional cells, however, p63 did highlight a preserved basal cell layer. Linear array analysis revealed the presence of HPV subtype 68.
Conclusions: We present a case of SCACP-IS which occurred spontaneously on the penis of an 83 year old man. This represents the first described case of in-situ SCACP occurring on the penis as well as the first reported case of SCACP of any kind to test positive for oncogenic HPV DNA.

521 Epidermotropic CD30 Positive Mycosis Fungoides (MF) with an Indolent Biologic Course.

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Background: The acquisition of a CD30 positive phenotype in a lesion of MF is often associated with transformation to a more aggressive neoplasm with adverse prognostic significance. Cases with isolated intraepidermal CD30 positive cells may bear a relationship to the Woringer-Kolopp form of pagetoid reticulosis, a generally indolent epidermotropic cutaneous T-cell neoplasm.
Design: We encountered four cases of atypical lymphoid infiltration characterized by a population of atypical intraepidermal CD30 positive cells. The light microscopic appearance was correlated with immunohistochemical results, molecular studies and clinical features.
Results: All cases showed a population of hyperchromatic CD30 positive lymphocytes in a distribution resembling pagetoid reticulosis. All cases had a significant reduction of CD7 expression and one showed deletion of CD5 in the atypical clone. The previous and subsequent biopsies demonstrated dermal nodules of atypical CD30 positive lymphocytes, histologically suggestive of lymphomatoid papulosis in two of four cases. In two of three cases where subsequent biopsies were performed, intraepidermal CD30 positive cells were absent. The clinical behavior of all 4 cases was that of an indolent process.
Conclusions: CD30 positive cells may be expressed as a transient dominant phenotype in cases of otherwise indolent MF with a pagetoid reticulosis morphology and appear to have no biological significance.

522 Distinctive Eosinophilic Cytoplasmic Inclusion Bodies in Melanocytic Nevi: An Immunohistochemical and Ultrastructural Study.

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Background: In 2004, Gottlieb noted cytoplasmic eosinophilic inclusion bodies in occasional melanocytes of both congenital and acquired nevi, stating that they may represent degenerative changes in an organelle, presumably melanosomes. Despite their distinctive features, no further pathologic findings have been formally reported. To our knowledge these inclusion bodies have not previously been described in Spitz nevi.
Design: Skin biopsies from 4 patients with known diagnoses of compound melanocytic nevus with congenital features (case No. 1, 2, and 3) or atypical Spitz nevus (case No. 4), all containing cytoplasmic eosinophilic inclusion bodies, were selected for histochemical, immunohistochemical and ultrastructural study. An additional 20 cases of malignant melanoma were examined for the presence of these distinctive cytoplasmic inclusions.
Results: In H&E-stained sections, the degree of density and eosinophilia of the cytoplasmic inclusion bodies varied with their size, smaller inclusions being more pale and homogenous, whereas larger inclusions showed a discernable dense core. Inclusions tended to be within multinucleate melanocytes featuring abundant vacuolated cytoplasm. They were ubiquitin-immunopositive and negative for tyrosinase, keratin, and vimentin. A panel of histochemical stains (PAS, Fontana, and Congo Red) showed no reactivity. Ultrastructurally, the inclusion bodies were nonmembrane-bound, ranged in size 4 to 7 μm and consisted of radiating filamentous structures with or without an electron dense core. Electron probe x-ray microanalysis showed no significant peaks when compared with tissue background. None of the malignant melanomas studied demonstrated inclusion bodies resembling those encountered in the present cases.
Conclusions: The inclusion bodies observed in our study do not resemble other cytoplasmic inclusion bodies histologically or ultrastructurally observed in melanocytic lesions. Being nonmembrane-bound, filamentous structure with or without an electron dense core, they were unrelated normal of degenerate melanosomes. We postulate they may be related to dysfunction of ubiquitin-mediated protein degradation occurring in melanocytes. Further studies focusing upon the composition and evolution of the inclusions may shed light upon their clinical significance and perhaps establish their utility in the differential diagnosis of melanocytic nevi and malignant melanoma.

523 TdT Expression in Rare CK20 Negative Merkel Cell Carcinoma.

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Background: Merkel cell carcinoma (MCC), also known as primary cutaneous neuroendocrine carcinoma, is an uncommon tumor of the elderly with indistinct clinical features and aggressive behavior. MCCs consist of small round blue cells making the diagnosis of MCC rather challenging and the use of immunohistochemistry (IHC) important. Typically, MCCs are positive for cytokeratin (CK) 20 in a paranuclear dot-like pattern and the neuroendocrine markers synaptophysin and chromogranin, and negative for CK7 and thyroid transcription factor 1 (TTF-1). Recent studies have demonstrated expression of the DNA polymerase terminal deoxynucleotidyl transferase (TdT) in MCCs. Herein we present a case series and immunophenotypic analysis of 5 patients with CK20 negative (CK20-) MCC.
Design: We reviewed a total of 40 MCC cases on file accessioned between 1995-2010 from the archives of Sunnybrook Health Sciences Centre. Only cases that had negative imaging studies with no evidence of a primary lung lesion were included. We identified a set of 5 MCCs with CK20- staining. All 5 cases were analyzed for clinical and pathologic attributes. Two pathologists and one dermatopathologist reviewed all the cases and studied the IHC profile using a panel of antibodies against: CK20, CK7, TdT, TTF-1, chromogranin and synaptophysin. Immunostaining was recorded semiquantitatively.
Results: The cases occurred in three males and two females, ranging in age from 74 to 82 years (mean 78.0). Four out of the 5 (80%) cases were from the lower extremities and the remaining case was a nodal metastasis. TdT staining was positive in 4 out of the 5 cases (80%) showing >25% of tumor cells reactive with moderate nuclear staining intensity. Two and all 5 MCCs showed moderate diffuse positive staining for chromogranin (40%) and synaptophysin (100%), respectively. All 5 cases were negative for CK20, CK7 and TTF-1.
Conclusions: Our study demonstrates that TdT is beneficial in rare cases of CK20- MCC.

524 Syringoid Eccrine Carcinoma: A Clinicopathologic and Immunohistochemical Study of 7 Cases.

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Background: Syringoid eccrine carcinoma (SEC) is an extremely rare malignant adnexal tumor of eccrine origin, often with variable presentations. We report seven cases of SEC, highlighting clinicopathological characteristics and immunohistochemical (IHC) features of these tumors.
Design: SEC cases accessioned between 2001-2010 were retrieved from the archives of the University Health Network. Seven cases were reviewed by three dermatopathologists and the IHC profile was examined using antibodies against: cytokeratin (CK) 5/6, CK7, CK14, CK20, low-molecular weight keratin (LMWK), high-molecular weight keratin (HMWK), epithelial membrane antigen (EMA), monoclonal carcinoembryonic antigen (mCEA), p63, estrogen receptor (ER), progesterone receptor (PR), androgen receptor (AR), S-100 and Ber-EP4.
Results: The cases occurred in three males and four females, ranging in age from 31 to 87 years (mean 58.9). Two of the lesions were from the head, three from the trunk and two from the upper extremities. All seven lesions were composed of an atypical infiltrative mass with syringoma-like tadpole morphology with ductular differentiation and prominent desmoplasia. Three cases demonstrated perineural invasion and two had positive lymph node metastases. Immunostaining was variable. Immunohistochemistry positivity was as follows: 6/7 cases were positive for CK5/6, CK7 (5/7), CK14 (3/4), CK20 (0/3), HMWK (2/4), LMWK (4/5), EMA (4/6), mCEA (4/5), p63 (3/5), ER (2/5), PR (1/4), AR (0/4), S-100 (2/5), and Ber-EP4 (3/3).

Conclusions: We believe the variability in both the histopathologic features and immunostaining seen in SECs is from this tumours ability to variably differentiate along multiple routes, including sweat secretory and/or ductal differentiation. Similar to other adnexal tumors, the role of myoepithelial cells within SECs is still controversial and may further contribute to the complexity and variability seen in these lesions. Future studies with new markers and discriminatory keratins may help elucidate SECs origin and differentiation.

525 Differentiating between Cutaneous Squamous Cell Carcinoma and Pseudoepitheliomatous Hyperplasia Utilizing QRT-PCR Analysis.

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Background: Squamous cell carcinoma (SCC) is one of the most commonly diagnosed cutaneous malignancies. The diagnosis can occasionally be challenging as there are many lesions that are clinical and pathologic simulators. One of the most challenging lesions to differentiate from SCC, especially in superficial and/or limited biopsy material, is pseudoepitheliomatous hyperplasia (PEH). The goal of our study was to develop a multiplex QRT-PCR-based assay which could be conveniently used in the clinical setting to distinguish between SCC and PEH.

Design: Thirty cases of PEH and 28 cases of SCC were identified from the Tamtron database and reviewed by the authors (SR and SB). The areas of interest on the paraffin blocks were isolated and the RNA was extracted and amplified. Select primers for the following genes: S100A7, S100A8, HOXC10, KRT9 and C15orf48 were utilized for QRT-PCR analysis. The genes chosen have shown differential expression between SCC and PEH from our DNA microarray studies.

Results: Multiplex QRT-PCR was able to accurately predict SCC and PEH in 54 of 58 cases (93%). The SCC was accurately determined in 27 of 28 cases (96%) and PEH in 27 of 30 cases (90%).

Conclusions: With further refinement of the test, this QRT-PCR-based assay can be used as a molecular diagnostic tool to distinguish between SCC and PEH in paraffin embedded tissue.

526 Aggressive Digital Papillary Adenocarcinoma: Clinicopathologic Study of 29 Cases of a Rare Neoplasm with New Observations.

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Background: Aggressive digital papillary adenocarcinoma (ADPA) was originally divided into benign and malignant categories based on histologic features. A later study defined a morphologic spectrum with a high recurrence and significant metastatic potential. All are now regarded as adenocarcinoma and with histologic features offering no distinction. There are only 2 large published studies.

Design: All cases with available slides diagnosed as ADPA were retrieved from our files (n=29). The slides were reviewed by two of the authors, and tumor morphology correlated with clinical features and outcome.

Results: Males were predominantly affected (n=28). Full clinical details were available for 27 patients. All involved a finger (n=23) or toe (n=4). Histopathologically, the tumor involved the dermis +/- subcutis. Epidermal hyperplasia (n=3), ulceration (n=2) and focal epidermal connection (n=1) were noted on occasion. The tumors had a lobular architecture (n=26) with a focally infiltrative pattern. They were unencapsulated with fibrous to sclerotic bands surrounding tumor lobules (n=17). Lesions were predominantly solid (n=23), with a cystic component ranging from <10% to 30% of observed tumor area. 6 had a cystic component comprising 50% or more of the tumour. Papillary projections were: prominent (n=9), focal (n=14), or not identified (n=6). Within the solid component, tubular structures were present at least focally in all cases. Focal keratinization was rare (n=2). Cytologic atypia ranged from mild to moderate (n=27), but was focally severe in 2 cases. Mitotic count ranged from <1 to 18 per square mm with focal necrosis in 6 cases. Stromal hyalinization was present at least focally in most cases, and was prominent centrally in some. 3 cases had prominent vascular dilatation with thrombi. Inflammation was not prominent and neurovascular invasion was not identified. All cases were treated by complete exicison; follow-up (n=18; range, 2m-10y) revealed local recurrence (n=3) and metastatic disease (n=3; lymph node in 1 and lungs in 2).

Conclusions: Recurrence rates in ADPA may not be as high as previously reported, although given the metastatic potential, they are best regarded as malignant and completely excised. ADPA can have a significant tubular/solid pattern with only a focal papillary component, and this may represent a pitfall in limited biopsies.

527 Expression of FOXP3 in HTLV-1-Associated Infective Dermatitissyndrome.

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Background: Human T-lymphotropic virus (HTLV)-1-associated infective dermatitis (ID) is a rare and severe chronic disease occurring mainly in children of certain areas of the world. These patients may subsequently present with cutaneous adult T-cell leukemia/lymphoma (ATLL) and HTLV-1-associated myelopathy (HAM)/tropical spastic paraparesis (TSP). The pathogenesis of ID remains undefined. Whereas it seems that regulatory T cells (CD4+CD25+FOXP3+ Tregs) represent the reservoir cells for HTLV-1 in ATLL and HAM/TSP, the status of Tregs in ID has not been reported to date.

Design: The immunohistochemical expression of FOXP3 was evaluated on sixteen skin biopsies from fifteen children and adults seen at the Dermatology Clinic, UPCH, Lima, Peru. All the patients fulfilled clinical and serological criteria for HTLV-1-associated ID. The histopathological findings of the biopsies were documented. FOXP3 status was analyzed in conjunction with patterns of expression of CD3, CD4, CD8, and CD25 by dermal and intraepidermal lymphocytes. Co-expression of CD4, CD25, and FOXP3 was assessed.

Results: The patients' age ranged from 5 to 82 years. Three histopathological patterns were identified. Some cases resembled seborrheic dermatitis, displaying spongiosis, parakeratosis, intracorneal neutrophils, and a superficial and deep perivascular lymphocytic infiltrate (7/16 cases). Other cases showed features of lichenoid dermatitis (8/16 cases). One case displayed a combination of the two previous patterns. All the cases showed intraepidermal lymphocytes, and in two cases, folliculotropism was identified. The presence of intraepithelial lymphocytes along with fibrosis of the papillary dermis (present in 6/16 cases) raised mycosis fungoides as a histopathological differential diagnosis. The lymphocytic infiltrate was mainly composed of CD3-positive T cells. All the cases showed a predominance of CD8-positive intraepidermal lymphocytes, whereas very rare CD4-positive cells were identified in 10/16 cases. CD25 was expressed in more than 10% of the cells in 4/16 (25%) cases; comparable FOXP3 expression was detected in 6/16 (37.5%) cases. Co-expression of CD25 and FOXP3 by more than 50% of the cells was seen in 14/16 cases.

Conclusions: Regulatory T cells (Tregs), defined as CD25+FOXP3+ T cells, are frequent in HTLV-1-associated ID and may correlate with the detected high numbers of CD8+ cells. Our results are in agreement with the postulate that early HTLV-1 infection stimulates the expression of FOXP3. Decreased expression of FOXP3, as reported in both HAM/TSP and ATLL, is not seen in ID.

528 Significance of Sentinel Lymph Node Isolated Tumor Cell Clusters in Overall Survival of Melanoma Patients.

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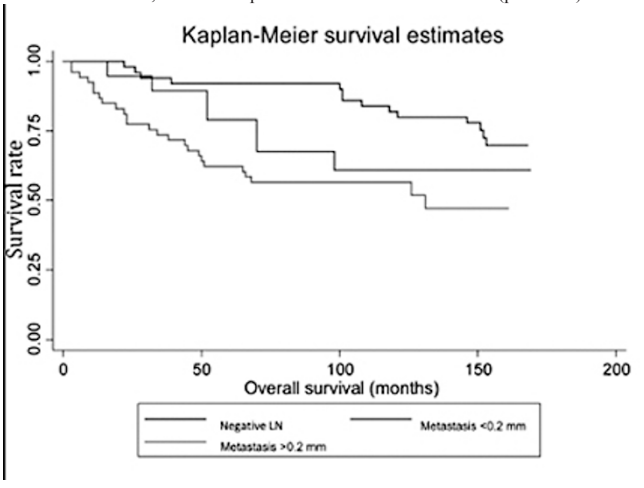
Background: Sentinel lymph node micrometastases are of prognostic significance in melanoma, and the 2010 AJCC classification cites no minimum volume criteria. Despite this recent codification, the prognostic significance of isolated tumor cells remains in question by many investigators and practitioners.

Design: Patients undergoing sentinel lymph node biopsy (SLNB) for Stage I/II melanoma at our institution between 1996 and 2005 were reviewed to identify patients with negative sentinel lymph nodes, those with micrometastases less than 0.2 mm in size, and those greater than 0.2 mm in size. Histologic and prognostic features of the primary melanoma were collected. The mean follow-up for patients was 4 years, with a median of 1.7 years.

Results: Of 122 patients who had a SLNB performed, 19 had micrometastases less than 0.2 mm in size in their SLN, and 53 had metastases greater than 0.2 mm. All microdeposits of melanoma stained positive with the melanocytic markers Melan-A and HMB-45 by immunohistochemistry. The Kaplan-Meier analysis of overall patient survival at 5 years was 94% in patients with negative SLN, 80% in patients with metastases <0.2mm, and 62% in patients with metastases>0.2mm (p=0.0037).



Conclusions: Patients with isolated tumor cells and very small deposits of melanoma less than 0.2 mm in size have a worse prognosis than patients with a negative SLNB. Accordingly, even isolated tumor cells in the lymph nodes of patients with melanoma should be interpreted as positive, allowing for additional therapeutic options including completion lymphadenectomy and experimental protocols.

529 GNAQ Gene Status in Cellular Blue Nevi, Spitzoid and Spindle Cell Melanomas.

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Background: Most benign and malignant melanocytic neoplasms display constitutive activation of the mitogen-activated protein (MAP) kinase pathway through mutations in BRAF, NRAS or HRAS genes. Recently, frequent somatic mutations of codon 209 (Q209L) in the heterotrimeric G protein a-subunit (GNAQ) gene have been reported in blue nevi (BNs) and uveal melanomas as an alternative route to MAP kinase activation. Since BNs are a heterogeneous group including common, cellular and atypical variants we aimed to investigate the differential presence of GNAQ activating mutations in BN

variants which has not been reported before. In addition, GNAQ status was investigated in desmoplastic melanomas which are often confused with BNs and in Spitzoid melanomas which usually lack BRAF mutations.

Design: The study series included 9 BNs (common variant -2, cellular variant -5, atypical -1), 4 desmoplastic melanomas and 4 Spitzoid melanomas. Following microdissection of tumor from the formalin-fixed paraffin-embedded material and DNA extraction, direct sequencing of exon 5 of GNAQ gene was performed and codon 209 was analyzed for presence of the mutation.

Results: The results of mutational analysis are summarized in the table below.

	Common BN	Cellular BN	Atypical BN	Spindle Cell/ Desmoplastic Melanoma	Spitzoid Melanoma
No. of cases	2	5	1	4	4
GNAQ mutation (%)	100%	0%	100%	0%	0%

$GNAQ^{Q209L}$ mutations were not found in any of the cellular BNs or melanomas but were present in the common BNs.

Conclusions: While to total number of cases analyzed is low, our results suggest that within the group of blue nevi, $GNAQ^{Q209L}$ mutations may only be present in the common form and are absent or rare in the cellular variant. This may indicate that the cellular variant of BN uses alternative mechanisms for MAP kinase activation that are different from those in common BN. In addition, no mutations were detected in the desmoplastic and Spitzoid melanomas analyzed.

530 Differential Expression of CCL5/RANTES in Alpha Beta vs. Gamma Delta Subtypes of Subcutaneous Panniculitis-Like T-Cell Lymphoma (SPTCL).

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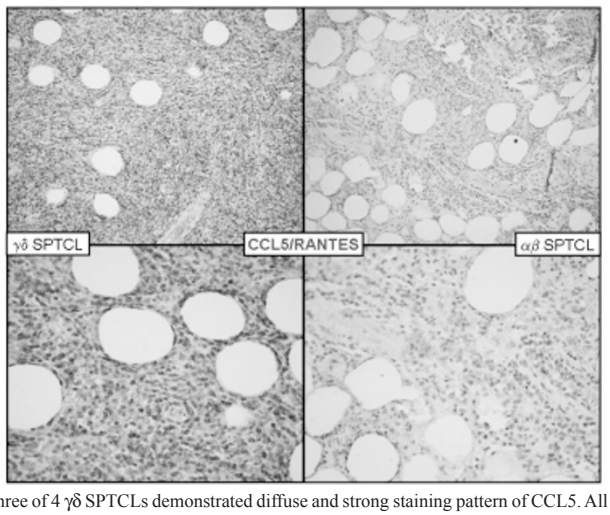
Background: SPTCL has 2 distinct subtypes, alpha beta (αβ) and gamma delta (γδ). The former is generally composed of $CD4^-CD8^+CD56^-\beta F1^+$ cells with a favorable prognosis and uncommon association with hemophagocytic syndrome (HPS), while the latter is of $CD4^-CD8^-CD56^{+/-}\beta F1^-$ T-cell phenotype with a poor outcome and frequent manifestation of HPS. The mechanism underlying the prognostic differences is unknown. Recent studies have shown that increased expression of Th1 chemokine, CCL5/RANTES is associated with pathogenesis and disease progression in obesity and atherosclerosis, via recruitment and activation of macrophages and T cells. Whether CCL5 plays a role in SPTCL is unclear.

Design: Skin biopsies were studied from 4 γδ and 3 αβ SPTCLs. IHC results of CCL5, selected T-cell markers and cytotoxic proteins were compared between the two forms of SPTCL.

Results: IHC results are shown below for γδ and αβ forms of SPTCL.

Table 1

Subtype of SPTCL	% of CCL5-Postive Cells	CD56	granzyme B	TIA-1	CD5	CD7	CD4/CD8
γδ	>90	+	+	+	-	+	-/-
γδ	>80	+	+	+	-	-	-/-
γδ	~30	-	+	+	+	+	-/-
γδ	>90	+	+	+	-	+	-/-
αβ	<25	NA	+	NA	+	+	⇓/+
αβ	<5	-	NA	+	+	+	-/+
αβ	<5	-	+	+	+	+	+/+



Three of 4 γδ SPTCLs demonstrated diffuse and strong staining pattern of CCL5. All 3 cases of αβ SPTCLs had significantly lower number of CCL5-positive cells with much decreased staining intensity. In 1 γδ SPTCL, ~30% of lymphoma cells were positive for CCL5. Interestingly, the lymphoma cells were $CD56^-$, while those of the other γδ SPTCLs were positive.

Conclusions: Chemokine CCL5/RANTES is diffusely expressed in malignant cells of γδ SPTCL but not in αβ SPTCL. Strong CCL5 expression is seen in lymphoma cells rimming adipocytes in γδ SPTCL. This expression pattern raises the possibility that CCL5 might play a role in adipotropism of SPTCL, similarly to its proposed role in adipose tissue inflammation associated with obesity. Specifically the ligand for CCL5, CCR5, is expressed by adipocytes. Further, the CCL5-CCR5 lymphocyte-adipocyte interaction may be associated with activation of macrophages, leading to hemophagocytosis and increased apoptosis, and thus the more aggressive clinical course seen in γδ SPTCL.

531 Five Cases of Indolent Gamma Delta T-Cell Lymphoma (TCL) Localized to the Subcutaneous Panniculus.

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Background: The WHO EORTC classification of lymphoma designates subcutaneous panniculitis-like T-cell lymphoma (SPTCL) exclusively to alpha beta (αβ) T-cell phenotype. Those of gamma delta (γδ) T-cell type are considered as other forms of primary cutaneous γδ TCL. The basis for this classification was the more indolent course of αβ form compared with the poor 5-year survival of γδ form. However, rare case reports exist of a waxing and waning clinical course in γδ form of SPTCL.

Design: Skin biopsies were studied from 5 patients with γδ TCL localized to the subcutaneous panniculus. A retrospective analysis of clinicopathologic features and follow-up was done.

Results: Clinical features of the 5 γδ TCLs are tabulated below.

Table 1

case	age at onset	sex	location	followup period	treatment	outcome
1	25	F	leg	7y	fludarabine	complete remission for 5y
2	33	F	NA	7y	CHOP + allo stem cell transplant	remission
3	36	F	arm thigh leg	7y	systemic chemo 5y after onset	died 7y after onset
4	76	F	axilla	0.5y	surgical resection with local radiation	remission
5	45	F	wrist leg chest	8y	fludarabin 6y after onset	died 8y after onset

y: years

Histologic features, IHC results of selected T-cell markers and cytotoxic proteins are shown too.

Table 2

case	dermal extension and adnexal involvement	adipocyte rimming	CD5	CD7	CD4/CD8	TIA-1/granzyme	CD56/CD62L
1	+	+	-	-	-/-	+	+/NA
2	+	+	-	+	-/-	+	-/-
3	+	+	-	+	-/-	+	+/-
4	+	+	-	+	-/-	+	+/NA
5	+	+	-	+	-/-	+	+/-

Case 3 was originally diagnosed as lupus profundus and treated with Plaquenil until 5y after onset. Earlier biopsies were better differentiated without the degree of hemophagocytosis or macrophage infiltration, although retrospective IHC studies revealed an identical phenotypic profile. Case 5 was originally interpreted as panniculitis and treated with antibiotics and steroids until 6y after onset. These 2 patients died 7 and 8 years after original presentation, due to complication of lymphoma and immunosuppression, respectively. Patient 5 was lymphoma-free at the time of death.

Conclusions: We present a series of 5 patients with γδ TCLs localized to the subcutis whereby the clinical course was indolent. Four were younger women with lesions in extremities, all of whom were alive 5y after presentation. All responded to chemotherapy. Complete remission was achieved in 4 cases. The exact pathogenetic mechanism that determines the outcomes in SPTCL is unclear. In this regard, the prior classification which grouped all primary subcutaneous T-cell lymphomas under SPTCL seems more reasonable.

532 Upregulation of Inhibitory Signaling Receptor Programmed Cell Death-1 (PD1) in Disease Evolution from Epitheliotropic T-Cell Dyscrasia (ETCD) to Mycosis Fungoides (MF).

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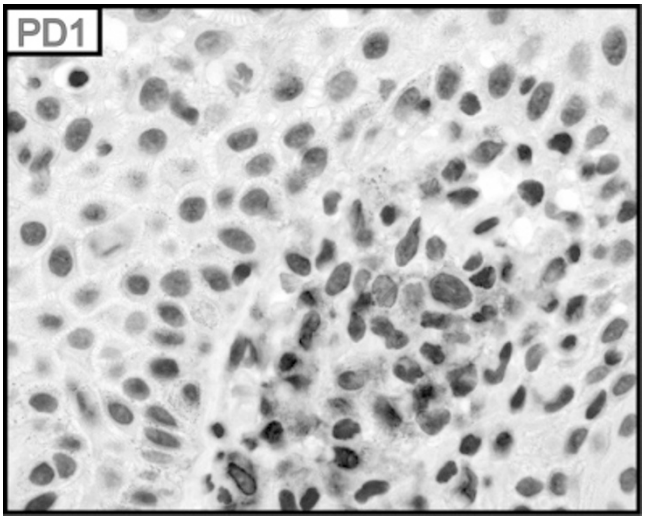
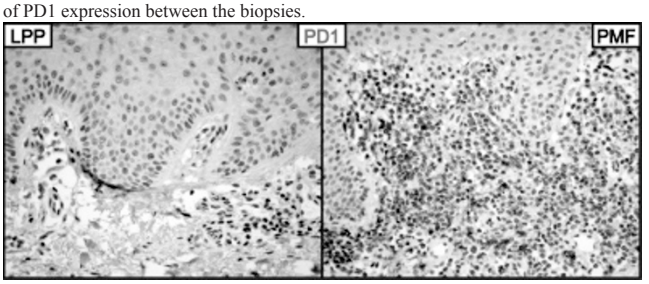
Background: Negative immunoregulatory checkpoints impede effective immune responses to tumor and reduce the action of anticancer agents. One such example is PD1, an inhibitory signaling receptor expressed on activated and CD4+CD25+foxp3+ suppressor T cells. PD1 ligand binding leads to inhibition of cytokine secretion by CD4+ T cells. Upregulation of PD1 has been suggested to be associated with disease progression in various tumors; however, whether PD1 plays a role in pathogenesis of MF is not clear.

Design: Skin biopsies were studied from 19 patients with MF, 6 with ETCD/large plaque parapsoriasis (LPP), and 1 with overlap features between LPP and early MF. IHC studies for PD1 and selected T-cell markers were performed, as well as T-cell clonality analysis.

Results: In 5/6 cases of ETCD/LPP, very few atypical lymphocytes exhibited PD1-positivity. In sharp contrast, focal strong staining of PD1 was observed in the neoplastic cells of 18/19 cases of MF.



The percentage of PD1-positive lymphocytes was also consistently higher in tumor stage than in patch and plaque stage MF. Interestingly, in the case where there was overlap between LPP and early MF, the number of PD1-positive lymphocytes approximated that seen in patch and plaque stage MF. Moreover, one patient presented skin biopsies at different stages of disease evolving from LPP to PMF, with a concomitant increase of PD1 expression between the biopsies.



Conclusions: Upregulation of PD1 correlates with disease progression in ETCD ranging from minimal staining in prelymphomatous dyscrasias to significant staining amidst neoplastic cells in more aggressive disease, likely reflecting the effects of PD1 on inhibiting tumor surveillance regulatory T cell populations. This observation thus raises the possibility to target PD1 for immunomodulation in MF therapy.

533 Molecular Diagnosis of Cutaneous Leishmaniasis and Species Identification: Analysis of 54 Histology Negative Skin Biopsies.

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Background: Cutaneous Leishmaniasis (CL) is endemic in the Middle East and North Africa and displays a wide spectrum of clinical manifestations. Confirming the diagnosis of CL histologically depends on the identification of the amastigotes, which may be inconclusive. The number of amastigotes may vary significantly depending on the strain type, host response & the disease stage. Accurate histological diagnosis is significant due to the requirement of targeted treatment.

Design: Skin biopsies from 122 patients from Lebanon, Syria, and Saudi Arabia with suspected untreated CL were reviewed. Clinical data includes age, gender, duration of the lesion, and biopsy type (shaved biopsy, SB versus punch biopsy, PB). Seven sections from each formalin fixed paraffin embedded skin biopsy (FFPE) were stained: 3 H&E, 1 Giemza, 1 AFB, 1 GMS & 1 PAS. All cases were reviewed by 2 pathologists and classified according to the modified Ridley's parasitic index (scale 0 to 6). DNA was extracted using a standard protocol from ribbons originating from the respective FFPE. PCR was performed using primers specific for the Leishmania ribosomal internal transcribed spacer 1 (ITS1-PCR). The digestion of the ITS1-PCR amplicons with the restriction enzyme *HaeIII* was performed for restriction fragment length polymorphism (RFLP) analysis and subsequent sub-speciation.

Results: Out of 122 skin biopsies, 54 cases (44.3%) showed a parasitic index of 0 to 1+ (no unequivocal amastigotes detected). Of the negative cases, 9 were SB and 45 were PB. The age ranged from 2 to 90 years (mean = 35 years, SD= 24.0 & males/females ratio is 6/5). The duration of the lesion ranged from 1 month to 60 months (mean= 10 months, SD= 14.6). ITS1 PCR was positive for all 54 cases (100% sensitivity). RFLP analysis identified *Leishmania tropica* sub-species in all cases.

Conclusions: Patients with clinically suspected CL, whose skin biopsies failed to detect Leishmania amastigotes, is a commonly encountered problem that represented 44.3% of the cases included in our study. The histologic confirmation of CL is crucial especially with the wide clinical manifestations and the availability of targeted therapy. In this study, we describe all stages of an optimized protocol from DNA extraction to sub-speciation by RFLP. According to our results, ITS1 PCR usage showed high sensitivity and specificity in confirming the diagnosis of CL where histology failed to detect Leishmania amastigotes.

534 Array Comparative Genomic Hybridization (aCGH) on Dermatofibromas (DF): Additional Evidence To Support a Neoplastic Process.

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Background: There has been considerable debate over the pathogenesis of DF. Some believe they represent a fibrosing inflammatory process, while others maintain they are neoplastic in nature. Reports of metastasizing DF, and the recognition of DF variants with a tendency to recur have fueled this debate, and catapulted it into the realm of clinical relevance.

Design: A total of 10 cases were studied: 8 DF (6 ordinary DF, 1 aneurysmal DF and 1 cellular DF); 1 angiomatoid fibrous histiocytoma and 1 hypertrophic scar. All cases were formalin-fixed paraffin-embedded. aCGH analysis of the lesional genome was performed using a 3039 probe whole genome bacterial artificial chromosome (BAC) microarray. The presence of copy number changes was correlated with additional clinico-pathologic findings, such as involvement of the subcutaneous tissue.

Results: Four cases showed copy number changes: The cellular DF showed the most obvious abnormalities with loss of large portions of 5q and 6q. Three ordinary DF showed other genomic abnormalities: One showed monosomy 19, a second showed a 19p loss, and a third showed a 19p gain. No obvious copy number changes were seen in any of the other four DF, in the angiomatioid fibrous histiocytoma, or in the hypertrophic scar. Of the eight DF studied, four showed focal involvement of the subcutaneous tissue and the other four were limited to the dermis. The four cases with the above described genomic abnormalities coincided with those showing focal involvement of the subcutaneous tissue.

Conclusions: The finding of copy number changes in a proportion of the cases studied suggests that DF, at least in some instances, may indeed represent a neoplastic process. The presence of genomic abnormalities in the subset of DF showing focal extension into the subcutaneous tissue, including the cellular DF, provides further evidence to support this hypothesis. Complete excision of dermatofibromas should be considered in those cases involving the subcutaneous tissue.

535 Immunohistochemistry for IgG4 on Paraffin Sections for the Diagnosis of Pemphigus.

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Background: Pemphigus is a group of autoimmune vesiculobullous diseases characterized by the presence of tissue-bound and circulating IgG antibodies directed against desmosomal adhesion proteins (desmoglein 1 and desmoglein 3) on the surface of keratinocytes. Both the IgG1 and IgG4 subclasses are produced, with IgG4 being predominant. Direct immunofluorescence (DIF) for IgG performed on fresh-frozen tissue plays a crucial role in diagnosing pemphigus. However, when paraffin sections of a biopsy specimen are histologically suspicious for pemphigus, frozen tissue may not be available to confirm the diagnosis. Immunohistochemical detection of total IgG performed on paraffin sections is of no diagnostic value because of the high background. In this study, we used immunohistochemistry for IgG4 performed on paraffin sections as a diagnostic test for pemphigus.

Design: Nineteen IF-proven pemphigus cases (12 pemphigus vulgaris, 6 pemphigus foliaceus, and 1 paraneoplastic pemphigus) were studied. Four normal skin specimens and 10 non-pemphigus vesiculobullous disease specimens served as controls. Paraffin sections of all cases were examined immunohistochemically for IgG4 expression. Positivity was defined as distinct, condensed, continuous immunoreactivity localized to the intercellular junctions of keratinocytes.

Results: The results were independently evaluated by three pathologists, with a 100% inter-observer agreement. Nine of 12 pemphigus vulgaris cases (sensitivity 75.0%), 4 of 6 pemphigus foliaceus cases (sensitivity 66.7%), and the paraneoplastic pemphigus case were positive for IgG4 immunohistochemical stain. The overall sensitivity was 73.7%. None of the control specimens showed IgG4 positivity (specificity of 100%), although non-specific staining was present in some cases. In the specimens demonstrating acantholysis, 8 of 10 pemphigus vulgaris cases (sensitivity 80.0%) and 4 of 4 pemphigus foliaceus cases (sensitivity 100.0%) were positive for IgG4. The overall sensitivity for specimens with acantholytic lesions was 86.7%.

Conclusions: Immunohistochemical labeling for IgG4 provides a sensitive and specific diagnostic tool for diagnosing pemphigus; it is likely to be particularly valuable in cases where frozen tissue is not available for DIF, and especially when active acantholytic lesions are examined.

Education

536 Clinical Relevance under the Microscope: Using Pathology To Stimulate Medical Student Motivation and Self-Regulated Learning in Histology.

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Background: Research suggests that medical students' motivation and interest for learning histology is low when it is not linked with relevant clinical applications. In this project, we studied the impact of an integrated teaching approach grounded in self-regulated learning (SRL) theory on student motivation and learning strategies in the histology laboratory. Using SRL as a theoretical framework, students are seen as active participants in constructing and assessing their own learning progress. The principal objective of this study was to determine how the use of an educational intervention