respectively. Among 23 HPV-negative HSIL patients, 21 (11 Cervista-negative and 10 HC2-negative) had histopathologic results within one year: CIN2/3 in 28.6%, CIN1 in 47.6%, and negative in 23.8%.

LSIL							
	HC2			Cervista			Average HPV
	Case#	Positive#	%	Case#	Positive#	%	positive (%)
	335	301	89.9	265	230	86.8	88.5
	1175	958	81.5	635	484	76.2	79.7
	354	268	75.7	237	165	69.6	73.3
Total	1864	1527	81.9	1137	879	77.3	80.2
HSIL							
	HC2			Cervista			Average HPV
	Case#	Positive#	%	Case#	Positive#	%	positive (%)
	25	25	100	19	16	84.2	93.2
	157	149	94.9	84	78	92.9	94.2
	34	30	88.2	13	11	84.6	87.2
	216	204	94.4	116	105	90.5	93.1

Conclusions: HC2 HPV-positive LSIL and HSIL rates were slightly higher than Cervista HPV-positive LSIL and HSIL rates, with only the significant difference in HPV-positive LSIL rate. Additional studies on positive and negative predictive values, sensitivity, and specificity are needed to fully assess differences in HPV test performance. Data in this study does not support allegations that HPV-positive rates with the Cervista method may be too high (Am J Clin Pathol 2010; 134:193-199).

446 HSIL Misinterpretation Papanicolaou Test Rates in the College of American Pathologists PAP Education and PAP Proficiency Test Program in 2013

Chengquan Zhao, Barbara Crothers, Mohiedean Ghofrani, Mojtaba Hussain, Fang Fan, Idris Ocal, Diane Davey. University of Pittsburgh Medical Center, Pittsburgh, PA; Water Reed National Military Medical Center, Bethesda, MD; PeaceHealth Lab, Vancouver, WA; University of Central Florida, Orlando, FL; Unisersity of Kansas Medical Center, Kansas City, KS; Mayo Clinic, Scottsdale, AZ; University of Central Florida College of Medicine, Orlando, FL.

Background: Misinterpretation of HSIL is an important problem in daily practice and in proficiency testing (PT). This study is to investigate variables related to misinterpretions of HSIL.

Design: We analyzed the 2013 CAP PAP Proficiency Test (PAP PT) and PAP educational programs (PAP-Edu) for misinterpreted HSIL cases and related variables, such as Pap preparation types, test types, and personnel.

Results: There were 26,122 responses for HSIL slides, including 11,397 in PAP PT and 14,725 in PAP Edu. Overall, 2672 (10.2%) responses for HSIL were misclassified as no response (0.2%), unsatisfactory (0.3%), negative (2.6%),or LSIL (6.9%). More CP slides were misclassified (12.3%) than ThinPrep (8.5%), but less than SurePath (14.7%; P=0.004). Cytotechnologists were significantly more likely to misclassify HSIL (11.5%) than pathologists (9.1%; $P < 0.001$) Overall, the misclassification rate for HSIL was lower in PAP PT (3.9%) than in PAP Edu (15.1%, P <0.001). The most common interpretation for a HSIL reference diagnosis was LSIL;negative or unsatisfactory interpretations accounted for 0.9% and 5.0% in PAP PT and PAP Edu, respectively. 2.6% pathologists and 5.5% cytotechnologists misclassified HSIL in PAP PT (P<0.001) and 14.4% pathologists and 15.9% cytotechnologists misclassified HSIL in PAP Edu (P<0.01).

Categories	Pap PT (%)	Pap Edu (%)	Total
Unsat	0 (0)	70 (0.5)	70
Negative	103 (0.9)	658 (4.5)	761
LSIL	343 (3.0)	1455 (9.9)	1798
HSIL	10950 (96.1)	12500 (84.9)	23450
No response	1 (0)	42 (0.3)	43
Total	11397	14725	26122

Table

Conclusions: LSIL is the most common misclassified category for HSIL. The concordance of pathologists for a HSIL interpretation is better than for cytotechnologists and may relate to differences in reporting responsibilities and PT grading criteria. SurePath had the highest misintepretation rate of HSIL. The HSIL misinterpretation rate is higher in PAP Edu than PAP-PT for both pathologists and cytotechnologists. This may represent a defensive strategy by participants in the test-taking environment, or reflect differences in selection criteria for slide validation and inclusion into PAP PT versus PAP Edu.

447 Cobas HPV Test Performance for High-Grade Squamous Intraepithelial Lesion (HSIL): Analysis of 130,648 Pap Tests With 1,654 Follow-Up Biopsy Cases With HPV Co-Testing

Haijun Zhou, Roxanne Mody, Eric Luna, Donna Armylagos, Mary Schwartz, Dina Mody, Yimin Ge. Houston Methodist Hospital, Houston, TX; University of Texas, Health Science Center, Houston, TX; BioReference Laboratories, Houston, TX.

Background: High risk HPV (HR-HPV) testing for ASC-US triage and co-testing with cytology has been implemented in clinical practice for many years. Recently, the Cobas HPV test was approved by the FDA as an option for primary cervical cancer screening in women over 25 years. However, clinical data of primary screening using the HPV test alone in the routine practice setting for detecting HSIL is currently lacking.

Design: Of 130,648 Pap tests performed at Houston BioReference Laboratories and Houston Methodist Hospital between March 1, 2013 and June 30, 2014, 51,315 had Cobas HPV co-testing or reflex for ASC-US. Of these 51,315 cases, 1,654 had follow-up biopsies. The cytologic and biopsy interpretations were rendered by board-certified cytopathologists or gynecologic pathologists at an academic medical center. All biopsies with interpretation of CIN2 were confirmed with p16/Ki-67 immunohistochemical stains. The HSIL reporting rate in this general screening population was 0.24% with an overall cytohistologic correlation rate of 70%. The majority of non-correlations (98%) were tissue sampling variances.

Results: In 1,654 cases with follow-up biopsies, the sensitivities of Cobas HPV test and Pap test to detect any dysplasia are 80.7% and 81.2%, respectively and the positive predictive values are 69.1% and 69.2%, respectively. For biopsy-confirmed high-grade cervical lesions (CIN2/3, AIS or Carcinoma, n=245), the negative rates of the Cobas HPV test were 9.4% and the cytology negative (NILM) diagnoses were 9.0%. Co-testing with cytology and the Cobas HPV test only missed 4 (1.6%) biopsy-confirmed CIN2+ cases.

Conclusions: In our study cohort, the Cobas HPV test alone is not superior to Pap test as a primary screening method for cervical dysplasia. The false-negative rate of the Cobas HPV test alone in detecting biopsy-confirmed high-grade cervical lesions is comparable to that of the Pap test. Co-testing with the Cobas HPV test and Pap test is the best strategy in detecting high-grade cervical lesions.

Dermatopathology

448 Dermal Malignant Peripheral Nerve Sheath Tumors Have a Distinctive Profile af DNA Copy Number Changes From Melanoma

Aleodor Andea, Min Wang, Gina Johnson, Rajiv Patel. University of Michigan, Ann Arbor, MI; Emory University, Atlanta, GA.

Background: Histological and immunophenotypic distinction of superficial dermal malignant peripheral nerve sheath tumors (MPNSTs) from desmoplastic melanomas lacking a junctional component is a notoriously difficult diagnostic distinction. Our aim in this study was to evaluate the spectrum of copy number variations (CNVs) and allelic imbalances in a series of dermal MPNSTs and compare them to published data in melanomas in a attempt to establish a differentiating profile.

Design: Stringent criteria were used to select unequivocal examples of dermal MPNSTs: the tumor had to arise in association with a nerve, in a patient with neurofibromatosis type I and lack a junctional melanocytic component. Three such cases of dermal MPNSTs were identified for the study. Following DNA extraction we performed comparative genomic hybridization using the Oncoscan V3 platform. Data was analyzed with Nexus Express for OncoScan software.

Results: Results are shows in table (* denotes CNVs shared with melanoma). All cases showed genomic loss involving the NF1 gene. One case had no additional abnormalities while the other two showed a complex karyotype. With the exception of losses on chromosomes 9, 10 and 13, the other abnormalities encountered are not commonly described in melanoma. Frequently occurring CNVs in melanoma such as gains of 1p12-31, 4q12-13.1, 5p, 6p, 7, 8q and 11q and losses of 6q and 17p were not detected.

Case	Gains	Losses	Copy Neutral LOH
1	1q21.1-44*, 9p11.2-q34.3, 16q21-24.3, 17q23.2-25.3	3p26.3-12.1, 4q34.3-35.2, 8p23.3-p11.1, 9p21.3*, 9p24.3-23, 9p21.2-13.2, 10p15.3-q11.21*, 11p15.4-11.12, 13q21.31-21.33*, 17q11.2-21.32, 20p13-p11.21, 21q21.1-22.3	7q11.1-11.21, 16q11.2-24.3
2	-	17q11.2	-
3	17q21.32-25.2, 22q11.1-13.1	1p36.33-31.3*, 2q24.1-37.3, 3p11.1-26.32, 7q11.22-22.1, 8p23.3-11.21, 9p24.3-21.1*, 11.15.4-11.12, 16p13.3-p11.1, 17q11.2, 20q12-13.12, 22q13.31-13.33	17q11.2-21.32

Conclusions: Our data suggests that while there is some overlap, the spectrum of CNVs in dermal MPNSTs is more akin to deeply seated MPNSTs and distinctive enough to allow separation from melanoma. Evaluation of CNVs in these lesions may have role as a diagnostic tool.

449 Evaluation of a New Gene Expression Assay Aimed To Differentiate Nevi From Melanomas

David Arps, Katherine Brick, Min Wang, May Chan, Rajiv Patel, Aleodor Andea. University of Michigan, Ann Arbor, MI.

Background: There are subsets of melanocytic lesions in which conventional histologic criteria fail to reliably differentiate nevi from melanomas. Current ancillary molecular studies aimed to help in the diagnosis of ambiguous melanocytic lesions are based on assessing DNA copy number alteration either by comparative genomic hybridization

or by FISH. Recently a new test based on expression levels of 14 genes with roles in immune signaling and differentiation has become commercially available. In an initial study this assay demonstrated a sensitivity and specificity of 90% and 91% respectively, in differentiating nevi from melanomas. Our goal was to evaluate this assay on a cohort of melanocytic lesions with various grades of atypia from one institution.

Design: 142 melanocytic lesions including 26 melanomas, 80 nevi, 34 ambiguous lesions and 2 reactive melanocytosis lesions were selected. Representative areas were marked on the HE sections and corresponding areas from unstained slides were micro-dissected. Following total RNA extraction the expression levels of 14 genes of interest and 9 housekeeping genes were determined by quantitative reverse transcription polymerase chain reaction. A score was computed based on the levels of expression with values <-2.1, -2 to -0.1, and >0 interpreted as benign, indeterminate and malignant, respectively.

Results: RNA was successfully extracted and amplified in 129 cases. Overall, the sensitivity and specificity in distinguishing invasive melanomas versus nevi without significant atypia was 83.3% and 96.8% respectively. The sensitivity for detecting melanoma in situ was 66.7%. The percentage of atypical (dysplastic) nevi with mild, moderate and severe atypia classified as benign by the assay was 87.5%, 75% and 45.5% respectively. All cases of Spitz, deep penetrating, blue, congenital, atypical genital and pigmented spindle cell nevi as well as the reactive melanocytosis lesions were classified as benign. For atypical Spitz nevi, 87.5 and 12.5% were classified as benign and indeterminate, respectively. The percentage of ambiguous cases classified as benign, indeterminate and malignant was 61.2%, 13% and 25.8%. Two cases of desmoplastic melanoma were classified as benign.

Conclusions: The assay performed well in differentiating various types of nevi including more unusual variants such as atypical Spitz nevi from melanoma. The performance declined for nevi with severe atypia (perhaps also as a reflection of more subjectivity in histological classification) and for desmoplastic melanomas.

450 Melanoma in Pregnancy: A 16-Year Clinicopathologic Review at a Single Referral Center

David Arps, Alison Durham, Timothy Johnson, May Chan. University of Michigan, Ann Arbor, MI.

Background: Melanoma accounts for 8-25% of all cancers diagnosed during pregnancy. Current literature suggests no significant role of pregnancy in the pathogenesis or prognosis of melanoma. We present our experience in melanomas diagnosed during pregnancy at a large referral center.

Design: The melanoma database at our institution was searched for melanoma cases diagnosed during pregnancy or within 6 months postpartum in 1998-2014. Clinical data were obtained from electronic medical records. Histologic slides of invasive melanomas were requested for microscopic review.

Results: Eighty-four patients (mean age, 30.7 years; range, 17-40 years) with confirmed pregnancy status at the time of melanoma diagnosis were identified. Fifteen of these cases (18%) were diagnosed as melanoma in situ (MIS) or evolving MIS, and the remaining 69 cases (82%) were invasive melanomas. The mean gestational age was similar in the two groups (20.5 and 19.5 weeks). One MIS and 5 invasive melanomas were diagnosed in the postpartum period. The most frequent sites of involvement were lower extremity (37%), back (19%), and upper extremity (13%). Up to 65% of patients with invasive melanoma noted change in a preexisting mole during pregnancy, whereas 21% reported the lesion as new. Invasive melanoma reportedly arose from a congenital nevus in 8% of cases, and from a longstanding nevus (since childhood) in another 10% of cases. 14% of patients with invasive melanoma developed metastatic disease. Review of the histologic data pertaining to invasive melanomas revealed the most common subtypes to be superficial spreading (64%), nodular (7%), and spitzoid (7%). Seven cases were diagnosed as melanocytic tumors of uncertain malignant potential (10%) in which invasive melanoma could not be excluded. The mean Breslow depth was 1.35 mm. Ulceration and mitoses were present in 12% and 48% of cases, respectively. Histologic evidence of a preexisting nevus was found in 42% of cases. Histologic slides were available for 26 invasive melanomas, of which 10 cases exhibited nevoid features of the invasive component.

Conclusions: A significant portion of pregnancy-associated invasive melanomas developed in preexisting nevi which were present at birth or since childhood. Further investigation into the specific effect of pregnancy on congenital nevi may lead to better understanding of the pathophysiology of melanoma associated with pregnancy.

451 Lymphovascular Invasion and BRAF Status in Primary Cutaneous Melanoma – Is There a Connection?

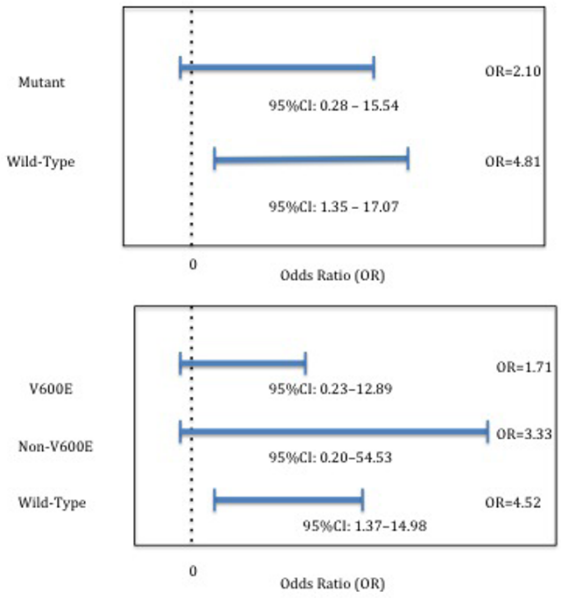
Phyu Aung, Dominick Leone, J Kyle Feller, Shi Yang, Joe Massaro, Marier Hernandez, Ron Yaar, Rajendra Singh, Thomas Helm, Meera Mahalingam. Boston University School of Medicine, Boston, MA; University of Texas MD Anderson Cancer Center, Houston, TX; Aurora Diagnostics, Greensboro, NC; Mount Sinai School of Medicine, New York, NY; New York State University, New York, NY.

Background: The interaction between BRAF status and lymphovascular invasion (LVI) in primary cutaneous melanoma (PCM) is unclear. Previously, we found a suggestive bivariate association between BRAF mutation and LVI, indicating that the predictors of LVI may be dependent upon BRAF status.

Design: A total of 103 PCMs were assessed for LVI (immunostaining with D2-40/S100 and H&E), and BRAF status (by DNA Sanger sequencing). Stratified multivariable logistic regression was used to determine effect modification by BRAF genotype.

Results: 31/103 samples exhibited a mutation: 19.4% V600E, 5.9% V600K, and 4.9% non-V600K. To increase power, variants of non-V600K and V600K were pooled together as "non-V600E mutants". None of the established histopathologic prognosticators were associated with LVI among either the non-V600E or V600E variants (OR=3.33, 95%CI: 0.20–54.53, p=0.39 and OR=1.71, 95%CI: 0.23–12.89, p=0.60 respectively); conversely, ulceration was associated with LVI among the wildtype (OR=4.52, 95%CI: 1.37–14.98, p=0.01). The association between ulceration and LVI depended on the presence of the BRAFWT allele (wildtype) even after mutation was further collapsed. Among samples carrying any BRAF mutation, there was no difference in the odds of detecting LVI in those with ulceration compared to those without ulceration (OR=2.10, 95%CI: 0.28–15.54, p=0.47); yet when examining only the wildtype, ulceration was associated with greater odds of LVI (OR=4.81, 95%CI: 1.35–17.07, p=0.02)

Figure 1: Association of LVI with Ulceration is Modified by BRAF Genotype -- (a) dichotomized (b) categorized by V600E status



Conclusions: Our findings suggest that BRAF mutational status may serve as an effect modifier in the relationship between LVI and ulceration. Similar associations may prove fruitful in personalized, genomic–based, therapy.

452 Actinic Prurigo Cheilitis: A Clinicopathologic Review of 75 Cases of Lip Lesions in Actinic Prurigo

Bjorn Batdorf, Victor Prieto, Patricia Mercadillo, Juan Carlos Diez de Medina, Silvia Lourenco, Martin Sangueza, Jose Plaza. Medical College of Wisconsin, Milwaukee, WI; University of Texas MD Anderson Cancer Center, Houston, TX; Hospital General de Mexico, Mexico City, Mexico; Hospital Obrero, La Paz, Bolivia; University of São Paulo, São Paulo, Brazil.

Background: Actinic prurigo is a chronic idiopathic photodermatosis that affects primarily American Indians in United States and Mestizos in Latin American countries. Clinically, the onset of the disease is usually in the first decade of life but may appear initially in adult life. It is characterized by symmetric involvement of sun-exposed areas of the skin, particularly areas of the face resulting in polymorphic erythematous papules, macules and plaques in different stages of evolution. Lower lip involvement includes swelling, scaling, fissures, hyperpigmentation and ulcerations of the vermilion border and in some cases could represent the only manifestation of the disease. The histopathologic features of actinic prurigo have been studied; however, it is unknown if actinic prurigo cheilitis without skin involvement has distinct histopathologic features that could allow accurate separation from other specific and non-specific forms of cheilitis.

Design: In this study we investigate the clinicopathologic features of 75 cases actinic prurigo cheilitis to provide further criteria for its diagnosis and classification. 33 cases were diagnosed as actinic prurigo cheilitis with cutaneous lesions and 42 cases were diagnosed as actinic prurigo cheilitis but without cutaneous lesions (only lip lesions).

Results: Histologically, of the 33 cases with actinic prurigo cheilitis with cutaneous lesions, 17 (52%) cases showed follicular cheilitis and 16 (48%) showed non-specific changes including spongiosis, acanthosis, exocytosis, eosinophilia and lichenoid changes. Of the 42 cases that had only lip lesions, 18 (43%) cases showed follicular cheilitis and 24 (57%) cases showed non-specific changes.

Conclusions: We have described the histopathologic features of actinic prurigo cheilitis without skin lesions to provide further criteria for its diagnosis. Follicular cheilitis is a distinctive and common histologic feature seen in actinic prurigo cheilitis with or without skin lesions.

453 A Landscape of Driver Mutations in Aggressive Digital Papillary Adenocarcinoma

Diana Bell, Victor Prieto, Doina Ivan. University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Aggressive digital papillary adenocarcinoma (ADPA) is a rare cutaneous adnexal neoplasm occurring on fingers, toes, palms and soles. They have an aggressive biological behavior with a relatively high potential for local recurrence (30-40%) and propensity for distant metastases (up to 14%). Complete surgical excision is the treatment of choice. However, to date, there are no uniform guidelines or recognized effective treatment for metastases. Targeted therapy may be a potential treatment option for these patients if a relevant oncogene mutation is identified. The aim of our study was to assess ADPA mutation status in an attempt to identify possible therapeutic targets not currently searched for in routine clinical practice. For this goal we performed a comprehensive genomic profiling of cases of ADPA from our database.

Design: Genomic DNA from 10 ADPAs was isolated from 5 µm-thick paraffin according to the manufacturer's protocol and submitted for the Sequenome MALDI TOF mass ARRAY platform. One microgram of genomic DNA per sample was submitted to the Sequencing and Microarray Facility at our institution. Each specimen was tested in duplicate for every mutation in the Sequenom panel.

Results: The Sequenome platform was used to profile 190 common oncogenic point mutations in 50 genes, and identified *BRAF-V600* mutations in 1 patient (10%). Other oncogenic mutations seen in other solid tumors (*EGFR*, *PIK3CA, RAS* (*KRAS, NRAS, HRAS*) and *MET*, activating the PI3K/AKT/mTOR and RAS/RAF/MEK pathways respectively were not identified in the ADPA cases examined.

Conclusions: Although only one patient of our initial ADPA series (10%) harbored one actionable alteration (BRAF-V600 mutation), this is for the first time identified and reported. A larger series of ADPA cases are currently undergoing genomic profiling. Given the limited treatment options and poor prognosis of patients with ADPA, this knowledge is clinically significant since patients with ADPA harboring this mutation may be offered BRAF-inhibitor therapies and completes an unmet clinical need.

454 Utility of Direct Immunofluorescence Testing for IgA in Patients With High and Low Clinical Suspicion for Dermatitis Herpetiformis

Scott Bresler, Scott Granter. Brigham & Women's Hospital, Boston, MA; Harvard Medical School, Boston, MA.

Background: In the current climate of increasing pressure to contain escalating health care costs and at the same time maintain excellent patient care, we undertook this study to compare the utility of direct immunofluorescence (DIF) testing for characteristic IgA deposits in patients with strong versus low clinical suspicion for dermatitis herpetiformis (DH). It is our experience that many dermatologists request DIF testing simply to rule out the disease in a patient without a particularly suggestive clinical picture.

Design: We searched the pathology records at our hospital for patients with suspected DH who underwent biopsy. We retrospectively analyzed the results of H&E and DIF testing in 77 cases and separated them into high and low clinical suspicion subgroups based on clinical findings and dermatologic impression.

Results: The overall sensitivity and specificity of H&E evaluation were 0.75 and 0.951, respectively, using a combination of DIF testing and clinical follow-up as the gold standard. The positive predictive value (PPV) and negative predictive value (NPV) were 0.8 and 0.935, respectively. Within the high clinical suspicion subgroup (36 cases), the sensitivity was 0.692 and the specificity was 0.957, compared to a sensitivity of 1.0 and a specificity of 0.947 for the low clinical suspicion subgroup (41 cases). In the high suspicion subgroup, the PPV was 0.9 and the NPV was 0.846. The PPV was 0.6 and the NPV was 1.0 for the low suspicion subgroup. Although histologic false negatives did occur, all were in patients within the high suspicion subgroup. Upon classification of each H&E diagnosis, the most common pattern observed was inflammation with eosinophils/hypersensitivity reaction (25 cases, 32.5%), followed closely by nonspecific chronic inflammation (23 cases, 29.9%). Stratification by clinical suspicion did not significantly alter this distribution.

Conclusions: In patients with a low clinical suspicion for DH, our data argue that it is reasonable to first perform a biopsy for routine histologic evaluation before requesting DIF analysis in selected patients. We show that the combination of low clinical suspicion and a negative H&E diagnosis result in a low probability of DH. In contrast, we found concurrent biopsy for routine microscopy and DIF testing in patients with high suspicion for DH will minimize false negative light microscopic findings. We believe these results will help to better triage patients for DIF testing.

455 Immunohistochemistry for 2SC and FH in Cutaneous Leiomyomas May Aid in Identification of Patients With HLRCC (Hereditary Leiomyomatosis and Renal Cell Carcinoma Syndrome)

Ben Buelow, Jarish Cohen, Nancy Joseph, Timothy McCalmont, Karuna Garg. University of California, San Francisco, CA.

Background: Presentation with multiple skin leiomyomas has been shown to be highly suggestive of Reed's syndrome (HLRCC), caused by germline mutations in the Fumarate Hydratase (FH) gene. Early identification of these patients is important since 20-30% develop aggressive renal cell carcinomas.

Bi-allelic inactivation of FH results in accumulation of 2-succinyl-cysteine (2SC). Antibodies to FH and 2SC have recently been developed and there are small studies assessing these stains in HLRCC associated renal tumors and uterine leiomyomas. However, these stains have not been evaluated in cutaneous leiomyomas.

Design: Patients with multiple (MCL) and single cutaneous leiomyomas (SCL) were identified from our dermatopathology archives from 1992-2014. We identified 22 patients with MCLs (40 total tumors) and selected 25 controls from patients with presumed SCLs. Patient age ranged from 21 to 91 (median 54.5). The H&E slides were reviewed and IHC for FH and 2SC were performed. Clinical information was extracted from electronic medical records.

Results: No morphologic differences were apparent between MLs versus SLs. Both 2-SC positivity and FH loss were strongly correlated with multiple leiomyomatosis by Fisher's exact test ($p<0.00001$ for both 2SC and FH, Table 1).

	Avg. age (std. error)	2SC positive	FH negative
SCL (25)	58.6 years (5.2)	2/25 (8%)	1/25 (4%)
MCL (40)	51 years (6.7)	37/40 (92.5%)	28/40 (70%)

Conclusions: We did not observe significant morphologic differences between MCL and SCL. MCLs are frequently positive for 2SC and negative for FH, while SCLs were typically 2SC negative and FH positive; our results further suggest that these stains, particularly 2SC, may be helpful in the identification of patients with MCLs who are at high risk for having HLRCC. The stains may be especially useful in cases with incomplete history or in patients with occult MCLs (in our study 8 of the patients with MCLs appeared to initially have SCLs until more detailed history was available). IHC for 2SC and FH could be considered in all cutaneous leiomyomas to help identify patients with HLRCC, providing an opportunity for the patient and their family members to have appropriate surveillance for renal tumors.

456 Next Generation Sequencing in the Diagnosis of Mycosis Fungoides: Performance as Assessed By Clinical Outcome

Eduardo Castro-Echeverry, Linden Watson, Kimberly Walker, Martin Fernandez, Arundhati Rao. Baylor Scott & White Memorial Hospital - Texas A&M Medicine, Temple, TX.

Background: The diagnosis of mycosis fungoides (MF) is challenging, requiring a combination of clinical follow-up, multiple biopsies, and correlation with immunohistochemical stains and molecular studies. Analysis for clonal T cell receptor (TCR) gene rearrangements using PCR amplification followed by fluorescence based sequencing (FBS) has limited sensitivity and specificity. Using clinical outcome as the gold standard, we evaluate the sensitivity and specificity of the LymphoTrack next generation sequencing (NGS) assay for monoclonal TCR gene rearrangements in the diagnosis of MF.

Design: The pathology database was queried for all skin cases where a TCR gene rearrangement assay was ordered between 2007 and 2013. Cases were reviewed to determine true positives and true negatives. True positives were defined as those which proved to be MF on clinical follow up. True negatives were defined as those where MF was suspected clinically or histologically, but which proved benign on clinical follow up. Formalin Fixed Paraffin Embedded (FFPE) tissue blocks were pulled and the LymphoTrack kit with 12 Ion Xpress™ indices and adapters were loaded on the Ion torrent chip and clonality assessed with NGS using the LymphoTrack Bioinformatics Software. Statistical analysis was performed using Microsoft Excel and STATA 11.

Results: Charts were reviewed for 201 cases, of which 40 cases were analyzed by NGS. Clinical follow up ranged from 6 months to 7 years. Sequence reads ranged from 3,776 to 531,621. Results were interpreted as polyclonal, oligoclonal, and monoclonal. The most common monoclonal gene rearrangements involved Vg4, Jg1/2. When oligoclonal results were considered negative, NGS had a sensitivity of 87% and a specificity of 94% for the diagnosis of MF. By contrast, the FBS assay showed a sensitivity of 80% and a specificity of 100% in this same subgroup. Several cases that were only suspicious for a monoclonal TCR rearrangement were found to have distinct monoclonal gene rearrangement by NGS. Those instances where the NGS and the FBS assay differed were either stable, early MF or persistent inflammatory lesions.

Conclusions: Using clinical follow up as the gold standard for MF, NGS was found to have a sensitivity superior to FBS with a lower specificity. Prospective studies are warranted to determine whether the specificity of FBS is superior, or if the negative cases identified by NGS as monoclonal are actually early MF.

457 Genomic Copy Number Analysis of Blue and Deep Penetrating Nevi Identifies Recurring Aberrations of Entire Chromosomal Arms in Malignant Blue Nevi

May Chan, Aleodor Andea, Paul Harms, Rajiv Patel, Min Wang, Patrick Robichaud, Gary Fisher, Timothy Johnson, Douglas Fullen. University of Michigan, Ann Arbor, MI.

Background: Blue nevi may display significant atypia (atypical blue nevi, ABN) or undergo malignant transformation (malignant blue nevi, MBN). Deep penetrating nevi (DPN) are another group of pigmented dermal melanocytic lesions characterized by variable degree of atypia. Morphologic distinction of benign cellular blue nevi and DPN from their atypical and malignant counterparts is notoriously difficult, and molecular tools are increasingly employed to aid in diagnosis.

Design: We studied copy number variations in 34 lesions from 34 patients including benign (conventional and cellular) blue nevi, ABN, MBN, benign DPN, and atypical DPN using Affymetrix's OncoScan 2.0 platform.

Results: Copy number aberrations were found in 0 of 5 benign blue nevi (0%), 4 of 11 ABN (36%), 5 of 10 MBN (50%), 1 of 5 benign DPN (20%), and 1 of 3 atypical DPN (33%) (see Table). Complex aberrations involving 5 or more regions were seen exclusively in MBN. Gains and losses of the entire short or long chromosomal arms were identified in 1 ABN, 1 atypical DPN, and 5 MBN. In particular, gains of 1q, 4p, 6p, and 8q, and losses of 1p and 4q were found in 2 or more MBN. Whole chromosome aberrations were also common, and represented the sole finding in 1 ABN and 1 benign DPN. When seen in MBN, however, whole chromosome aberrations were invariably accompanied by partial aberrations of other chromosomes.

	Gain	Loss
ABN #1	15q11.2-13.1, 15q13.1-26.3	15q13.1
ABN #2	15q21.2-25.3	-
ABN #3	1q, 8q	1p, 3
ABN #4	20	-
MBN #1	1q, 7, 15q11.2-26.3, 20	1p, 2, 4, 5q31.2-35.3, 9, 16, 18, 17q11.2-21.31
MBN #2	1q32.3-44, 6p, 8q21.3-24	1p, 3p, 9p
MBN #3	1q, 4p, 8q	1p, 3, 4q, 8p, 9p21.3-22.2
MBN #4	1q, 4p, 8q	1p, 3, 4q
MBN #5	6p	-
Benign DPN #1	-	9
Atypical DPN #1	-	6q, 9q

Conclusions: Our study shows that copy number variations involving entire chromosomal arms are common in MBN. Some changes are shared by both ABN and MBN, however the latter is more likely to harbor additional aberrations, suggesting progression in tumor biology. As most of the recurring regions identified in this study are not targeted by conventional melanoma FISH probes, development of new probes targeting these regions will likely improve detection of MBN. Our data concur with previous studies in that genomic aberrations are relatively infrequent in benign and atypical DPN.

458 Utility of CD123 Immunostain in the Distinction of Cutaneous Lupus and Cutaneous T-Cell Lymphoma

Stephanie Chen, Alexandra Hristov, May Chan. University of Michigan, Ann Arbor, MI.

Background: Cutaneous lupus erythematosus (LE) may display a robust intraepidermal lymphocytic infiltrate simulating epidermotropism in mycosis fungoides (MF). Conversely, MF may demonstrate interface change resulting in diagnostic confusion with LE. Also well documented is the histologic overlap between lupus profundus (LuP) and subcutaneous panniculitis-like T-cell lymphoma (SPTCL). Clusters of CD123+ plasmacytoid dendritic cells (PDCs) are common in LE and LuP, however the density and distribution of PDCs in cutaneous T-cell lymphoma have not been characterized.

Design: Skin biopsies diagnosed as LE (n=18), LuP (n=12), MF (n=25), and SPTCL (n=5) were retrospectively reviewed and immunolabeled with CD123 antibody. The percentage of CD123+ cells of the entire infiltrate was estimated from 0 to 100% in 10% increments. The presence of any clusters consisting of at least twenty CD123+ cells, as well as any intraepidermal CD123+ cells, were noted.

Results: The results are summarized in the Table below. Significant differences were identified between LE and MF, in that LE showed a higher density of CD123+ cells ($p<0.0001$), frequently comprising at least 20% of the entire infiltrate ($p=0.0029$) and forming clusters ($p<0.0001$). Intraepidermal CD123+ cells, however, were common in both LE and MF ($p=0.4048$). A statistically significant difference was found between the mean percentages of CD123+ cells in LuP versus SPTCL ($p=0.0460$). However, no significant difference was detected between these two entities by using a 20% cutoff ($p=0.1088$), or by the presence of intraepidermal CD123+ cells ($p=0.8997$).

	LE (n=18)	LuP (n=12)	MF (n=25)	SPTCL (n=5)
Mean age (years)	47	46	72	74
No. of cases with interface change	18 (100%)	7 (58%)	13 (52%)	0
Mean % CD123+ cells	21%	18%	4%	0 (rounded)
No. of cases with at least 20% CD123+ cells	12 (67%)	6 (50%)	3 (12%)	0
No. of cases with CD123+ cluster(s)	17 (94%)	8 (67%)	0	1 (20%)
No. of cases with intraepidermal CD123+ cells	18 (100%)	2 (17%)	19 (76%)	1 (20%)

Conclusions: CD123 immunostaining is a helpful ancillary tool in differentiating LE from MF. On the other hand, the utility of CD123 in the distinction of LuP and SPTCL is limited, with the caveat of relatively small sample sizes in this study.

459 Next Generation Sequencing of Cytokeratin-Negative Merkel Cell Carcinoma

Angela Collie, Paul Harms, Andi Cani, Rajiv Patel, Daniel Hovelson, Michaela Haller, Douglas Fullen, Steven Billings, Scott Tomlins. Cleveland Clinic, Cleveland, OH; University of Michigan, Ann Arbor, MI.

Background: Merkel cell carcinoma (MCC) is a rare but highly aggressive cutaneous neuroendocrine carcinoma. Cytokeratin-20 (CK20) is expressed in approximately 95% of cases of MCC and is useful for distinction from morphologically similar entities including metastatic small cell lung carcinoma. Lack of CK20 expression may make definitive diagnosis of MCC more challenging. The biological significance of CK20 negativity in MCC is unknown. In approximately 80% of cases, conventional (CK20-positive) MCC is associated with the oncogenic Merkel cell polyomavirus (MCV). MCCs lacking MCV display distinct genetic changes from MCV-positive MCC, including *RB1* inactivating mutations. Unlike conventional MCC, most CK20-negative MCC are MCV-negative, suggesting that CK20-negative MCC predominantly arise through virus-independent pathway(s). This observation raises the possibility that CK20-negative MCC harbor further genetic differences from conventional MCC.

Design: We analyzed 16 CK20-negative MCC tumors (10 MCV-negative, 4 MCV-positive, 2 MCV unknown) using the Ion Ampliseq Comprehensive Cancer Panel, which provides data on genome-wide copy number changes and coding mutations in over 400 cancer-relevant genes.

Results: 13 tumors displayed chromosomal gains or losses, averaging 4.4 copy number changes per tumor. Recurrent copy number changes included gains of chromosome 1 and loss of chromosomes 11 and 17. Mutations were detected in 15 tumors, with an average of 12.9 prioritized high confidence non synonymous somatic mutations per tumor (in 403 genes analyzed). Genes with recurrent tumor suppressor mutations included *TP53* (8/16, 50%), *RB1* (3/16, 19%), *TSC1* (2/16, 13%), and *BAP1* (2/16, 13%). Of these, all but *TP53* mutations were restricted to MCV-negative tumors. *PIK3CA* mutations were found in 3/16 (19%), including hotspot activating mutations E542K and H1047L, and were restricted to MCV-negative tumors. Additional oncogenes with activating mutations in MCV-negative tumors were *AKT1* (E17K) (1 tumor) and *EZH2* (Y641F) (1 tumor). No oncogenic hotspot mutations were detected in MCV-positive tumors.

Conclusions: CK20-negative MCC display overlapping genetic changes with conventional MCC, including mutation of *TP53* and *RB1*. However, a subset of CK20-negative MCC harbor mutations not previously described in conventional MCC, including oncogenic activating mutations in *AKT1* and *EZH2*, and recurrent inactivating mutations in *BAP1*. Hence, CK20-negative MCC harbor diverse oncogenic drivers which may represent therapeutic targets in individual tumors.

460 A New FISH Panel and Next Generation Sequencing for the Diagnosis of Melanocytic Lesions

Kristine Cornejo, Xun Wu, Melissa McEnery-Stonelake, Xiuling Meng, Keith Tomaszewicz, Matthew Welch, Ediz Cosar, April Deng, Lloyd Hutchinson. University of Massachusetts Medical School, Worcester, MA.

Background: Malignant melanoma (MM) is the most deadly of all skin cancers. Its one of the most common cancers among young adults with a fast growing incidence. The histopathological diagnosis of MM can be very challenging as a variety of benign melanocytic lesions share morphology with MM. A false negative diagnosis can delay treatment critical for preventing metastasis, but a false positive diagnosis can result in unnecessary therapy. We employed molecular methods to develop an assay that can assist with the clinical diagnosis of MM.

Design: Fourty-five cases (MM, n=26; benign nevi, n=19) were analyzed with multiplex ligation-dependent probe amplification (MLPA) of 103 cancer genes (~[/underline]200 probes) to discover gene copy number changes that distinguish benign nevi (BN) from MM. Matched normal tissue from the same specimen was used for comparison. MLPA results were confirmed by fluorescence in situ hybridization (FISH) using a 10 probe panel and in a subset by Next generation sequencing (NGS) copy number analysis. Genes with confirmed gains or losses ≥40% were then used to create a 4-color FISH panel. This panel was compared to published FISH probe sets. BRAF, NRAS, KRAS and KIT point mutations were investigated using PNA clamp-real time quantitative PCR and/or Sanger sequencing and a subset were confirmed by NGS.

Results: One MM and 5 BN failed at least one assay. MLPA studies identified gene copy number differences in the following genes with commercial FISH probes: CCND1, MYB, PIK3CA, CDKN2A, PTEN, BRAF, KIT and MYC. FISH analysis showed one BN had a hemizygous deletion of CDKN2A. All other BN were normal. Genes most frequently altered in MM were RREB1, BRAF and MYC (~80% each). The NGS copy number assay agreed with FISH results. Our 4-color FISH panel (CDKN2A, PIK3CA, PTEN, BRAF) and the published FISH probe set (RREB1, CCND1, CEP6, MYB) distinguished BN from MM with a sensitivity/specificity of 91%/92% and 92%/100% respectively. A combined panel based on average copy number per cell >2.5 or <1.5 for RREB1, MYC, PTEN and BRAF had a sensitivity and specificity of 100%. Sequencing showed BRAF mutations in BN and MM (86% vs 28%) but only MM showed NRAS (8%), KIT (8%), or KRAS (4%) mutations and this was mutually exclusive.

Conclusions: FISH offers a practical approach to looking for chromosome abnormalities that distinguish BN from MM. NGS shows promise in MM diagnosis given that a combined analysis for mutation and copy number changes may be better for the diagnosis of MM.

461 The Role of Sentinel Lymph Node Biopsy in Cutaneous Adnexal Carcinomas: The MD Anderson Experience With 348 Cases

Richard Danialan, Kudakwashe Mutyambizi, Phyu Aung, Victor Prieto, Doina Ivan. University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Cutaneous adnexal carcinomas (CAC) are rare tumors with varying lines of differentiation, a relatively high local recurrence rate, and the propensity to metastasize to lymph nodes and distant organs. To date, complete surgical excision is the treatment of choice. The value and prognostic information of sentinel lymph node biopsy (SLNB) is well-established for tumors such as breast carcinoma and melanoma. However, the role of SLNB in CAC is not yet clearly defined. The goal of our study was to assess in a large case series the potential utility of SLNB in CAC as a possible predictive factor of patient outcome.

Design: 348 cases of CAC diagnosed or treated in our institution between 1995-2014 were selected from our database, including 53 porocarcinomas (PC), 57 hidradenocarcinomas (HAC), 41 digital papillary adenocarcinomas (DPAC), 197 sebaceous carcinomas (SC). SLNB was performed for 89 patients: 16 with PC (30%), 16 with HAC (28%), 9 with DPAC (22%), 48 with SC (24%).

Results: PC and DPAC showed the greatest affinity for SLN involvement: 9 (56.25%) for PC, 4 (44%) for DPAC. SLNB was positive in 4 cases (25%) of HAC and 14 cases (29%) of SC. The patients' follow-up ranged from 3 months to 11 years. Some patients with positive SLNB died of disease: 2 (50%) with HAC, 3 (33%) with PC, 2 (14%)

with SC. Patients with SC, PC and HAC and negative SLNB were alive and without metastatic disease at their follow-up. Interestingly, all patients with DPAC and positive SLNB survived but 3 (9%) of DPAC patients for which SLNB was not performed presented with lung metastases and died of disease.
Conclusions: This is the largest case series on SLNB in CAC to date. No well-established or uniform guidelines are available and SLNB in such cases is dependent on the clinical judgment. In our institution, SLNB was offered for 89 (25.6%) of patients and a total of 31 patients had positive SLNB. Some of the subtypes of CAC, such as PC and DPAC, had a greater propensity to involve SLN. A significant number of patients with positive SLNB, especially with PC, SC, and HAC, died of disease. We have not yet identified a clear correlation between positive SLNB in patients with DPAC and their outcome. We are currently assessing histologic parameters in the primary tumor that may potentially be associated with positive SLNB and predict a poorer prognosis. In conclusion, especially in certain subtypes of CAC, SLNB may be considered as a potential predictive factor of patients' outcome and trigger a longer and closer clinical and radiological follow-up to detect possible recurrences.

462 Elafin Is a Likely Immunomarker To Differentiate Between Skin Graft-Versus-Host Disease and Erythema Multiforme on Routine Skin Biopsies

Maria De la Garza Bravo, Rebecca Penland, Patricia Chevez-Barrios. Houston Methodist Hospital, Houston, TX.
Background: Graft-versus-host disease (GVHD) is one of the principal causes of morbility following an allogenic hematopoietic stem cell transplantation (HSCT) and can affect three target organs: skin, liver, and the gastrointestinal tract. Due to its accessibility, skin biopsies to confirm a GVHD are frequently performed. Of several available diagnostic biomarkers elafin, a serine protease inhibitor, has been identified as the best single diagnostic GVHD discriminator. GVHD is not the only condition that leads to skin reactions in post-transplant patients. Erythema multiforme (EM) can occur due to drug reactions, infections, and/or physical factors. Since both reactions display similar histopathological, novel immunohistochemical (IHC) markers are needed. In the present study we evaluated, for the first time, the ability of elafin to distinguish between GVHD and EM skin lesions.
Design: Clinical and histopathologic features of 11 GVHD and 10 EM/EM-like patients seen from 2009-2013 were reviewed. Seven patients with normal skin (NSk) biopsies were used as controls. All diagnostic skin biopsies were analyzed by IHC using a polyclonal elafin antibody. Elafin immunoreactivity was characterized based on its epidermal site (spinous, granular, spinous+granular, or granular+corneal), pattern (linear or patchy), and intensity (1-3+).
Results: We reviewed a total of 37 biopsies (12 GVHD, 15 EM/EM-like, and 10 NSk). None of the patients with EM/EM-like lesions had a history of HSCT, and all except one showed a possible drug association. All GVHD patients had a previous history of HSCT. Of 12 GVHD lesions, 10 skin biopsies displayed a positive elafin staining in the granular layer (linear pattern,Fig.1C-D). Conversely, the EM/EM-like biopsies revealed mixed elafin epidermal immunoreactivity, with only a single biopsy showing a granular pattern similar to GVHD (Fig.1A-B). All normal skin biopsies show no immunoreactivity to elafin (Fig.1E-F).

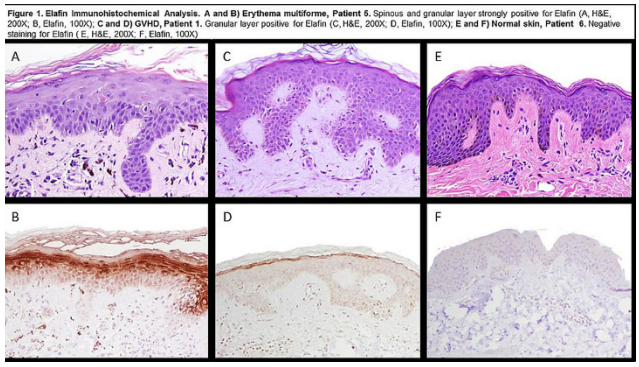


Figure 1. Elafin Immunohistochemical Analysis. A and B) Erythema multiforme, Patient 5. Spinous and granular layer strongly positive for Elafin (A, H&E, 200X; B, Elafin, 100X). **C and D) GVHD, Patient 1.** Granular layer positive for Elafin (C, H&E, 200X; D, Elafin, 100X); **E and F) Normal skin, Patient 6.** Negative staining for Elafin (E, H&E, 200X; F, Elafin, 100X)

Conclusions: In this study we have shown that elafin is a promising immunomarker in distinguishing GVHD from EM on routine skin biopsies with a particular emphasis on post-transplant patients.

463 Lipid Poor Xanthogranuloma – Review of 98 Cases

Olena Dorokhova, Bijal Amin, Mark Jacobson. Montefiore Medical Center and Albert Einstein College of Medicine, Bronx, NY.
Background: Lipid-poor xanthogranuloma (LPX) is an underrecognized variant of conventional 'juvenile' xanthogranulomas (JXG). We present a case series of LPX to define the clinicopathologic spectrum of this entity.
Design: Retrospective review of 92 cases diagnosed as LPX from 2 medical centers.
Results: Mean age - 31 years (range 2 month - 74 years). M:F ratio - 1.7:1. Clinical differential diagnoses ranged from benign cyst and verruca to melanoma and lymphoma. Distribution of lesions: head and neck – 36, lower extremities - 20; upper extremities - 18; trunk - 24. Histologically the LPXs could be divided into 3 types: epithelioid cell, spindle cell and mixed. The lesions were composed of epithelioid and/or spindle cells arranged diffusely throughout the dermis, an increased number of small blood vessels and an admixed inflammatory cell infiltrate with lymphocytes, plasma cells and eosinophils; only rare cells had hints of lipid within their cytoplasm, mitoses were common. Histological differential diagnoses included lipid poor xanthogranuloma, reticulohistiocytoma, melanoma, Langerhans cell histiocytosis, infection, poorly differentiated carcinoma, and lymphoma. In 54 cases the diagnosis of LPX was rendered based on H&E alone. In 44 cases immunohistochemistry (IHC) was performed with 4 immunostains per case on average (range 1 - 15 markers). IHC results: CD68 - positive in 43 cases (diffuse in 42, focal in 1); S100 - negative in 35 and focally positive in 6 lesions; MelanA - negative in 32 cases; pancytokeratins - negative in 7 lesions; factor XIIIa - negative in 2 and focally positive in 16; HAM56 - positive in 7 cases; CD4 - positive in 2; CD31 - positive in 1 case and negative in the other; CD34, CD30, tyrosinase and CD1a were negative in all cases they were performed (6, 2, 4 and 13 cases, respectively); vimentin was positive in one and negative in the other. GMS, AFB and/or PAS stains were performed in 6 cases and were negative. No recurrences have occurred to date.
Conclusions: We outline the constellation of clinical and morphologic features of LPX which is probably a rapidly growing variant of conventional JXG. Recognition of LPX by general pathologists and dermatopathologists is critical as this entity is often not suspected clinically and histologically may simulate malignancy.

464 Epithelioid Fibrous Histiocytoma Is Characterized By ALK Rearrangement and Overexpression

Leona A Doyle, Adrian Marino-Enriquez, Christopher Fletcher, Jason Hornick. Brigham & Women's Hospital and Harvard Medical School, Boston, MA.
Background: Recurrent fusions involving *protein kinase C* and genes encoding membrane-associated proteins have recently been reported in benign fibrous histiocytoma (FH). *ALK* rearrangement was recently described in 2 cases of FH with epithelioid features, with corresponding ALK expression detectable by immunohistochemistry (IHC). The goal of this study was to determine the frequency of ALK expression by IHC in epithelioid fibrous histiocytoma (EFH; also known as epithelioid cell histiocytoma), to determine its sensitivity and specificity for the diagnosis of EFH compared to other FH variants, and to correlate ALK expression with *ALK* gene rearrangement in EFH by fluorescence in situ hybridization (FISH).
Design: ALK protein expression was evaluated in whole tissue sections from 29 EFH and 41 other variants of FH (11 conventional and 10 each cellular, atypical and aneurysmal types). Cases of EFH were typically well-circumscribed and composed of a monotonous population of polygonal cells with palely eosinophilic cytoplasm, minimal nuclear atypia, frequent binucleate cells, and prominent thin-walled vessels. IHC was performed following pressure cooker antigen retrieval using a mouse anti-ALK monoclonal antibody (clone 5A4; Leica). FISH was performed using Vysis LSI *ALK* dual color break-apart probe (Abbott Molecular) on 4-µm sections on 12 EFH with ALK expression (1 technically unsuccessful), and on 3 cases without (2 technically unsuccessful).
Results: In total, 25/29 cases (86%) of EFH showed diffuse cytoplasmic expression of ALK by IHC. Expression was moderate to strong in intensity in all cases except one, which showed diffuse weak expression. All other types of FH were negative for ALK by IHC. FISH demonstrated *ALK* rearrangement in all IHC-positive cases successfully examined by FISH (n=11); in contrast, *ALK* rearrangement was not seen in 1 ALK-negative EFH.
Conclusions: ALK expression is present in the majority (86%) of EFH and is associated with *ALK* gene rearrangement. ALK expression is not seen in other variants of FH, suggesting that EFH is an unrelated, biologically distinct tumor type. ALK expression may be useful in distinguishing EFH from histologic mimics.

465 Methylation Analysis of the TERT Promoter in Pediatric Melanoma

Yiping Fan, Gang Wu, Seungjae Lee, John Morris, Peter Vogel, Reinhard Dummer, Alberto Pappo, Raymond Barnhill, Armita Bahrami. St. Jude Children's Research Hospital, Memphis, TN; University Hospital of Zurich, Zurich, Switzerland; Institut Curie, Paris, France.
Background: Thestabilization of telomeric DNA, which secures immortality, is essential for the development of overt malignancy. In both adult and pediatric conventional melanoma (CM), telomeres are sustained by increased transcriptional activity of *telomerase reverse transcriptase* (*TERT*), often as a result of mutations in the *TERT* promoter (*TERT*-p). However, the molecular mechanism underpinning telomere maintenance in melanomas arising in giant congenital nevi (CNM) has not been elucidated. An alternative mechanism of TERT expression in many cancers is by *TERT*-p methylation. We sought to determine the methylation status of the *TERT*-p in subtypes of pediatric melanoma by conducting a genome-wide DNA methylation analysis.
Design: Genomic DNA was extracted from FFPE tissues of 22 melanocytic lesions (20 pediatric and 2 adult cases): 7 CM, 6 spitzoid melanoma (SM) with favorable behavior, 4 fatal SM (2 pediatric and 2 adult cases), 3 CNM, and 2 giant congenital nevi (GCN). Samples were analyzed by PCR and direct sequencing of a portion of *TERT*-p. For methylation analysis, 500ng of DNA was bisulfite converted using the Zymo EZ DNA Methylation Kit and processed for hybridization on the Illumina HumanMethylation450 BeadChip. Beta values representing the fraction of methylated cytosine present at each CpG site were calculated after normalizing the signals and adjusting type2 probe bias. For 7 samples (1 CNM; 4 SM; 2 CM) with RNAseq data, the gene expression value was calculated by HTSeq. In addition, RNA in situ hybridization (ISH) for TERT (RNAscope®) was used to examine the expression of TERT in 2 CNM and 2 GCN.
Results: A mutation in the *TERT*-p was found in all CM (3 SNV 280; 3 SNV 250; 1 SNV 242/243) and fatal SM (3 SNV 280; 1 SNV 242/243), but in none of the GCN, CNM, or SM with favorable behavior. Methylation analysis showed that *TERT*-p CpG at the ch5:1295737 position (575 bps upstream of transcription start site) was completely methylated in the 3 CNM, while it was partially methylated in the CM and SM samples, and completely unmethylated in the 2 GCN. The methylation level at this position correlated positively with TERT expression (r^2=0.265). RNA ISH of TERT showed high-resolution signals in 2 CNM and no signals in 2 GCN.

Conclusions: Methylation profiling identified a distinct DNA methylation pattern of the *TERT*-p in CNM that correlated positively with TERT expression. Our findings strongly suggest that TERT-p methylation is the underlying molecular mechanism for TERT expression in CNM, which is distinct from that in CM and true SM.

466 Prognostic Significance of Lymphovascular Invasion Detected By immunostaining With D2-40 and D2-40/MITF1 in Patients With Cutaneous Melanoma

Laurence Feldmeyer, Patricia Fox, Michael T Tetzlaff, Jonathan Curry, Priya Nagarajan, Doina Ivan, Carlos Torres-Cabala, Victor Prieto, Phyu Aung. University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Lymphovascular invasion(LVI) is considered by some authors a major determinant of adverse outcome in primary cutaneous melanoma(PCM), however, it is not included as an essential criterion in current AJCC/CAP staging/treatment. The incidence of LVI in PCM assessed on hematoxylin and eosin(H&E) staining alone ranges from 0-6%, but it increases with the use of adjuvant immunohistochemistry(IHC) for endothelial cells. It is interesting that the reported incidence of LVI in PCM is disproportionate to that of sentinel lymph node(SLN) involvement, which ranges from 19-47%. This discrepancy may be due to the difficulties associated with ascertaining LVI by H&E alone. Therefore, we examined the role of LVI detected by IHC in the prognostic assessment of melanoma patients using a large sample size.

Design: We performed a retrospective analysis of 120 patients with PCM seen at MDACC(1/09-7/14). The presence or absence of LVI, as determined by IHC for D2-40 only or double staining for D2-40/MITF1(DS), was compared to H&E and correlated with histological, demographic and clinical outcome parameters, including SLN involvement.

Results: LVI in PCM was assessed using DS(n=56) or D2-40(n=64) and H&E(120). The use of IHC endothelial markers significantly increased the detection rate of LVI. A significant number of patients reported negative for LVI on H&E were positive for LVI when assessed with DS(21/55=38%, $p<0.01$) or D2-40 alone(13/60=22%, $p=0.0003$). For DS, significant associations of LVI were observed with increasing Breslow thickness and mitotic figures($p<0.03$, both). For D2-40, in addition to association with Breslow thickness and mitotic figures($p<0.01$, both), we noted positive correlation with ulceration($p<0.01$) and negative correlation with radial growth phase($p=0.02$). The presence of LVI was also associated with increased rate of SLN metastasis; the odds of SLN positivity in LVI positive patients was 5.2(DS) and 26(D2-40) times greater than those of LVI negative patients($p<0.01$, both). In D2-40, the presence of LVI was significantly associated with satellite/in transit metastasis($p=0.02$).

Conclusions: Our results indicate that IHC increases LVI detection in PCM and that LVI is significantly associated with adverse clinicopathologic parameters, especially SLN metastasis. All cases positive for LVI(except one), as detected by IHC had SLN metastasis. Therefore we propose the use of IHC as a routine method for evaluation of LVI in PCM cases for prognostic purposes and to consider LVI as a positive predictive marker for SLN metastasis.

467 Expression of the High-Affinity Nerve Growth Factor Receptor TrkA in Cutaneous Malignancies With a Propensity for Perineural Invasion – Is There a Connection?

Noah Frydenlund, Dominick Leone, Brendon Mitchell, Ossama Abbas, Meera Mahalingam. Boston University School of Medicine, Boston, MA; American University of Beirut Medical Center, Beirut, Lebanon.

Background: Tropomyosin receptor kinase A (TrkA) is a tyrosine kinase that serves as the high-affinity receptor for nerve growth factor (NGF). While *ex vivo* studies have shown TrkA to be associated with perineural invasion (PNI) in non-cutaneous malignancies, literature on TrKA and PNI in cutaneous neoplasms is limited to only one study. Given this, our aim was to investigate TrkA expression in cutaneous lineage-unrelated malignancies with a propensity for PNI and to evaluate the relationship between TrkA and select histopathological prognosticators.

Design: In this IRB approved study, archival tissue samples were obtained from the pathology files of the Skin Pathology Laboratory, Boston, MA. Lineage-unrelated malignancies with a propensity for PNI included cutaneous SCC of the head & neck (H&N) area (n=57) and desmoplastic melanoma (n=45). Controls for these were non-H&N cutaneous SCC (n=53) and non-desmoplastic melanomas (n=47) respectively. As TrkA is a membrane bound receptor, only samples with immunohistochemical membranous TrkA staining in >25% of cells were deemed positive. Histopathologic prognosticators evaluated included depth, Clark Level, degree of differentiation and morphological subtype (latter two in SCC alone). Pearson correlation and Stuarts's Tau-c were used to assess the relationship between TrkA and PNI, bivariate associations were examined using Fisher's $\mathbf{c}^2$ test.

Results: PNI was detected in 23% of SCCs from the H&N area, 16% of SCCs from non-H&N areas, 62% of desmoplastic melanomas and 0% of non-desmoplastic melanomas. In SCCs, TrkA expression was 2.90 times more likely to be observed in H&N areas compared to non-H&N (28/57 versus 12/48, *p=0.01)*. In Melanomas, no TrkA expression was seen in either group. In SCCs, a strong positive correlation was noted between TrkA expression and PNI (r=1.0, p<.001) regardless of site and expression was associated with decreased degree of differentiation (OR=6.46, p=0.0006) and high-risk variants (OR = 6.53, p=0.002).

Conclusions: TrkA expression correlates with PNI, and up-regulation in SCCs alone indicates that expression is lineage-related. *In vitro* studies have shown that UV-exposure down-regulates TrkA expression resulting in apoptosis in normal keratinocytes. Given this, up-regulation of TrkA in SCCs from sun-exposed areas, in particular in poorly differentiated tumors and high-risk variants, suggests a role for the NGF-TrKA axis in tumorogenesis.

468 Perineural Invasion in Cutaneous Squamous Cell Carcinoma – an Immunohistochemical Study of Correlation With Anatomic Site and Established Histopathologic Prognosticators

Noah Frydenlund, Dominick Leone, Brendon Mitchell, Ossama Abbas, Meera Mahalingam. Boston University School of Medicine, Boston, MA; American University of Beirut Medical Center, Beirut, Lebanon.

Background: Perineural invasion (PNI) has been recently added to the AJCC squamous cell carcinoma (SCC) staging criteria as a high-risk tumor characteristic and is purportedly more common in cutaneous SCC of the head and neck (H&N) area. However, substantial variation in the reported incidence of PNI exists, likely related to the challenges of identifying PNI based on H&E stained sections alone. Given this, we developed a double immunostaining (DIS) protocol with the primary aim of more accurately ascertaining the incidence of PNI. Secondary aims were to determine correlations between DIS detected PNI and established histopathologic prognosticators (tumor depth, Clark level, degree of differentiation and high-risk morphologic variant) in cutaneous SCC.

Design: In this IRB approved study, archival tissue samples with a diagnosis of cutaneous SCC were obtained from the pathology files of the Skin Pathology Laboratory, Boston, MA. A total of 57 cases from the H&N area were identified as meeting criteria for study inclusion. The control group comprised cutaneous SCC from non-H&N areas (n= 53). Double immunostaining (DIS) was performed using antibodies to S100 and p63 for identification of nerve and tumor cells respectively.

Results: UsingH&E, PNI was detected in 6/57 (11%) cases from the H&N area and 3/53 (6%) cases from non-H&N areas ($p=0.49$). Using DIS, PNI was detected in 13/57 (23%) of SCCs from the H&N area and 8/53 (16%) of SCCs from non-H&N areas ($p=0.30$). Overall, there was significant disagreement between PNI determined by H&E and by DIS ($\kappa=0.47$, $p=0.002$).The incidence PNI as detected by DIS was 1.41 times greater in SCCs of the H&N area compared to those from non-H&N areas, although this increase was not significant ($p=0.56$). Of the histopathologic prognosticators evaluated, only tumor depth was significantly associated with PNI ($p=0.002$); there was a linear trend showing increasing depth correlates with PNI ($p=0.008$).

Conclusions: Findings from the current study, the first to use DIS for identification of PNI in cutaneous SCC, indicates that the incidence of PNI in the H&N areas is not significantly different from that in non-H&N areas. The observed increase in PNI detection with DIS and its correlation with select histopathologic prognosticators, underscore the utility of immunohistochemistry as a useful adjunct in microstaging.

469 Immunohistochemistry for CD117 Is Effective in Distinguishing Cutaneous Adnexal Tumors With Apocrine/Eccrine or Sebaceous Differentiation From Other Non-Merkel Cell Epithelial Tumors of the Skin

Keisuke Goto. Kainan Hospital, Yatomi, Japan.

Background: In normal skin tissue, CD117 (c-Kit) protein is expressed in melanocytes, mast cells, and skin adnexa especially in the eccrine units. The present study sought to confirm the effectiveness of CD117 immunostaining for diagnosing cutaneous adnexal tumors.

Design: Immunostaining with an antibody against CD117 (polyclonal, A4502, dilution 1:200; Dako) was performed for representative sections of 79 clinicopathologically confirmed cutaneous apocrine/eccrine tumors, 9 sebaceous tumors, 47 follicular tumors, 51 keratinocytic tumors, 23 metastatic tumors of known origin, and 1 mammary Paget disease. This study did not include any cases of Merkel cell carcinomas, melanocytic lesions, hematological tumors, or mesenchymal tumors. CD117 staining detected not only in the cytoplasm but also in the cell membrane of ≥5% of the tumor cells was considered positive. Staining of 5–50% of the tumor cells was considered focal, and staining of >50 to 100% diffuse. The intensity of positive staining was semi-quantitatively graded as weak, moderate, or strong.

Results: The results are summarized in the following table. Seventy-five (95%) of the 79 apocrine/eccrine tumors were positive for CD117, but only 1 mucinous carcinoma, 1 apocrine carcinoma, and 2 extramammary Paget disease were negative for CD117. For most positive cases, the staining was diffuse (57/75 cases) and moderate to strong (67/75). Eight (89%) of the 9 sebaceous tumors were CD117-positive, showing mainly focal (7/8) and weak (6/8) positivity. Of the 47 follicular tumors, all 4 cases of pilomatricoma and 4 other cases were positive for CD117. Of the 51 keratinocytic tumors, only 2 cases were positive. None of the 23 metastases or the mammary Paget disease was positive.

Tumor type	Positivity	Extent	Intensity
Tumors with apocrine/eccrine differentiation	**75/79 (95%)**	**F18, D57**	**W8, M27, S40**
Syringofibroadenomatous hyperplasia	1/1 (100%)	F1, D0	W1, M0, S0
Hidrocystoma	5/5 (100%)	F0, D5	W0, M1, S4
Hidrocystadenoma	2/2 (100%)	F1, D1	W0, M1, S1
Poroma	29/29 (100%)	F7, D22	W3, M8, S18
Hidradenoma	9/9 (100%)	F2, D7	W0, M7, S2
Hidradenoma papilliferum	1/1 (100%)	F0, D1	W0, M0, S1
Spiradenoma	3/3 (100%)	F0, D3	W0, M0, S3
Cylindroma	1/1 (100%)	F0, D1	W0, M1, S0
Syringoma	1/1 (100%)	F0, D1	W1, M0, S0
Mixed tumor of the skin	15/15 (100%)	F4, D11	W0, M5, S10
Porocarcinoma	2/2 (100%)	F2, D0	W2, M0, S0
Ductal carcinoma	1/1 (100%)	F0, D1	W0, M1, S0
Cribriform carcinoma	1/1 (100%)	F0, D1	W0, M1, S0
Adenoid cystic carcinoma	1/1 (100%)	F0, D1	W0, M0, S1
Endocrine mucin-producing sweat gland carcinoma	1/1 (100%)	F0, D1	W0, M1, S0
Mucinous carcinoma	0/1 (0%)		
Apocrine carcinoma	0/1 (0%)		
Extramammary Paget disease	2/4 (50%)	F1, D1	W1, M1, S0
Tumors with sebaceous differentiation	**8/9 (89%)**	**F7, D1**	**W6, M2, S0**
Sebaceoma	3/3 (100%)	F3, D0	W3, M0, S0
Sebaceous carcinoma	5/6 (83%)	F4, D1	W3, M2, S0
Tumors with follicular differentiation	**8/47 (17%)**	**F6, D2**	**W5, M3, S0**
Trichofolliculoma	0/2 (0%)		
Trichilemmal cyst/Proliferating trichilemmal cyst	0/5 (0%)		
Desmoplastic trichilemmoma	0/1 (0%)		
Inverted follicular keratosis	0/4 (0%)		
Pilomatricoma	4/4 (100%)	F3, D1	W3, M1, S0
Trichoblastoma/Trichoepithelioma	1/5 (20%)	F1, D0	W0, M1, S0
Basal cell carcinoma	3/20 (15%)	F2, D1	W2, M1, S0
Keratoacanthoma	0/6 (0%)		
Tumors with keratinocytic differentiation	**2/51 (4%)**	**F2, D0**	**W2, M0, S0**
Seborrheic keratosis	0/15 (0%)		
Actinic keratosis	1/8 (13%)	F1, D0	W1, M0, S0
Bowen disease	1/13 (8%)	F1, D0	W1, M0, S0
Squamous cell carcinoma	0/14 (0%)		
Metastatic carcinomas	**0/23 (0%)**		
Mammary Paget disease	**0/1 (0%)**		

D, diffuse (> 50%); F, focal (5-50%); M, moderate; S, strong; W, weak

Conclusions: It was demonstrated that immunohistochemical CD117 expression is specific for cutaneous adnexal tumors with apocrine/eccrine and sebaceous differentiation as well as pilomatricomas in non-Merkel cell epithelial tumors of the skin. CD117 could be a useful marker for the differential diagnosis of cutaneous adnexal tumors especially for distinguishing apocrine/eccrine or sebaceous tumors from follicular, keratinocytic, or metastatic tumors.

470 Holocrine Poroma: A Distinctive Entity From Eccrine and Apocrine Poroma

Marcelo Horenstein, Marina Sandoval, Jack Jacob. The Dermatology Group, West Orange, NJ; New York Institute of Technology College of Osteopathic Medicine, Old Westbury, NY.

Background: As early as 1984, complex poroma-like adnexal lesions have been variably characterized as showing sebaceous and apocrine-like differentiation. Since then, 28 cases have been reported by Harvell, Zaim, Hanau, Gianotti, Groben, Lee, Santos-Briz, Misago, and Kazakov. Here we add 32 cases.

Design: Two board-certified dermatopathologists reviewed 86 poroid tumors and recorded clinical and histologic features. In addition we performed immunohistochemistry (IHC) and statistical analysis (SA).

Results: 51 cases had classical features of eccrine poroma (EP). 3 cases had apical decapitation secretions characteristic of apocrine poroma (AP). 32 cases had complex histology with basaloid and squamous cells, cytoplasmic vacuolization, sebocytes, ducts with cuticles, and holocrine secretions, which we categorized as holocrine poroma (HP). In Table 1 we list differential characteristics of HP vs EP.

	HP (n=32)	EP (n=51)	SA
Age (yr)	64.0	60.0	NS
Gender (male)	13 (40.6%)	28 (54.9%)	NS
Location (head and neck)	23 (71.9%)	11 (21.6%)	p
Size (mm)	4.1	6.7	p
Compound (epidermal and dermal)	24 (75.0%)	37 (72.5%)	NS
Ductal formation (0-4)	2.5	2.4	NS
Stromal hyalinization (0-4)	1.0	2.7	p
Vacuolization (0-4)	1.6	0.4	p
Follicular structures (0-4)	1.4	0.0	p
Squamous differentiation (n)	32 (100%)	1 (2.0%)	p

We found no atypical cells, infiltration, or clinical evidence of Muir Torre syndrome in HP. IHC in 23 selected HP cases with CK903 scored 4/4, CAM 5.2 scored 0.6/4, EMA scored 1.9/4, CEA scored 1.6/4. We found no loss of MLH-1, MSH-2 or MSH-6 in any of the 11 HP cases tested.

Conclusions: Complex poroid lesions have a definitive association with the sebaceous duct, therefore we labeled them as holocrine poroma. HP characteristically have a heterogeneous cytology including basaloid and squamous cells, and ductal differentiation, evidenced by ducts lined by steatocystoma-like cuticles and containing holocrine secretions. HP is variably associated with follicular and sebaceous structures. HP has no evidence of eccrine or apocrine differentiation. HP can be differentiated from EP by their heterogeneous histologic features, head and neck distribution, smaller size, presence of follicular, squamous and sebaceous elements, and by their lack of stromal hyalinization.

471 Expression of Master Regulators of Mature T-Cell Differentiation FOXP3, T-Bet, and GATA-3 in Cutaneous Adult T-Cell Leukemia/Lymphoma By Immunohistochemistry

Andy Hsi, Gregory Brown, Chyi-Chia Lee, Andras Schaffer. Washington University School of Medicine, St. Louis, MO; National Institutes of Health, Bethesda, MD.

Background: Adult T-cell leukemia/lymphoma (ATLL) is a rare and aggressive mature T-cell malignancy caused by the human T-cell lymphotropic virus type 1 (HTLV-1). Approximately one half of ATLL patients show cutaneous manifestations, which are histopathologically identical to those seen in mycosis fungoides. The majority of non-cutaneous ATLL cases are characterized by the CD4+CD25+FOXP3+ regulatory T-cell (Treg) phenotype, while mycosis fungoides expresses markers of helper T-(Th) cell differentiation with T-bet (Th1) and GATA-3 (Th2) positivity in early and late stages, respectively. The expression pattern of T-cell transcriptional master regulators FOXP3, T-bet and GATA-3 in cutaneous infiltrates of ATLL is unknown.

Design: Eleven cases of cutaneous ATLL were included in this study. Seven cases were of early stage disease showing superficial dermal involvement with 2 cases showing epidermotropism, while 4 cases were of late stage disease with diffuse dermal/subcutaneous infiltrates. All cases were stained with antibodies against CD25, FOXP3, T-bet, and GATA-3. The proportion of neoplastic cells with CD25, FOXP3, T-bet, or GATA-3 positivity was analyzed. Positive expression is defined as >20% staining for a given marker.

Results: Membranous CD25 was positive in all cases (100%). Nuclear FOXP3, contrary to prior non-cutaneous studies, was positive in only 2 early stage cases (18%), predominately in the epidermotropic neoplastic cells. Nuclear T-bet expression was mixed, with 6 cases (55%) of early and late stages showing positive expression and 5 cases (45%) with negative staining. Nuclear GATA-3 was diffusely present in all cases (100%), with weak staining intensity in early stage and strong intensity in late stage disease.

Conclusions: In contrast to non-cutaneous HTLV-1 transformed T-cells, ATLL cells in the skin appear to exhibit complex T cell differentiation profiles with a Treg/Th2, Th1/Th2 or Treg/Th1/Th2 phenotype in early and a Th2 or Th1/Th2 phenotype in late stages. Thus, an immunohistochemistry panel of FOXP3, T-bet, and GATA-3 may help with the differential diagnosis of cutaneous ATLL and primary cutaneous T-cell lymphomas.

472 There Is Not a Clinical Utility for Granulysin as a Prognostic and Diagnostic Marker in Tissue Sections for SJS/TEN

Kelli Hutchens, Daniel Cramer, George Garib, Michael Mosier, Stephanie Kliethermes, Richard Gamelli. Loyola University Medical Center, Maywood, IL; Loyola University, Chicago, IL.

Background: Steven's Johnson Syndrome and Toxic epidermal necrolysis (SJS/TEN) are life threatening mucocutaneous sloughing disorders with marked epidermal/ mucosal necrosis. The necrosis is T cell-mediated where cell death is induced by CD8+ cells; however the exact mechanism of cell death is unknown. Granulysin is a cytolytic molecule released by CD8+ T cells that has been implicated as a mediator of SJS/TEN with some evidence for a role in prognosis. We aim to determine if granulysin immunohistochemical (IHC) may be used clinically for either prognosis or diagnosis of patients with suspected SJS/TEN.

Design: 89 samples from patients who presented with concern for SJS/TENS over 3 years were evaluated. 52 SJS/TEN, 9 EM, 28 other dermatitides. Microarrays were made and paraffin sections were double stained with granulysin antibody (Anti-Granulysin RF10 Mouse IgG) and CD8 antibody (Anti-CD8 Mouse IgG-DAB). 3 High power

fields (HPF) were counted to obtain a ratio of granulysin stained cells to CD8 cells = granulysin density (GD). Diagnosis, septic events, oral and ocular involvement, mortality, LOS, and SCORTEN were recorded and compared to GD.
Results: There was no correlation between GD and LOS, septic events, mortality, or SCORTEN (p=0.47). There was no difference in GD between SJS/TEN and EM. The mean GD was significantly higher for SJS/TEN (0.3927) than for all other dermatitis (0.2947), (p=.0096). Nonspecific dermatitis and antibody mediated dermatitis had very low GD.

Non-SJS/TEN Dermatitis	Granulysin Density	Previous Reported Association with Granulysin	Epidermal Necrosis Present
LCV	0.014359	-	-
Spongiotic Dermatitis	0.09722	-	-
Bullous Pemphigoid	0.11671	-/+	-
Pemphigus	0.21316	-	-
Pustular Dermatitis	0.27977	-	+/-
Erythema Multiforme	0.39902	+/-	+
Drug Eruption	0.44195	+/-	+/-
Folliculitis	0.48518	+	+/-
Lichenoid Dermatitis	0.50927	_	+

Conclusions: Our data corroborates the role of granulysin in epidermal necrosis as GD increased in both clinically benign and severe progressive dermatologic conditions with histologic necrosis. Contrary to studies on blister fluid granulysin levels, we see no clinical utility for granulysin IHC for early identification or risk stratification of patients with cutaneous sloughing disorders.

473 BRAF Fusions Are Characteristic Spitzoid Melanomas

David Jones, Andrew Carlson, Kai Wang, Ashley Tarasen, Christine Sheehan, Siraj Ali, Julia Elvin, Juliann Chmielecki, Roman Yelensky, Doron Lipson, Vincent Miller, Philip Stephens, Jeffrey Ross. Albany Medical College, Albany, NY; Foundation Medicine Inc, Cambridge, MA.
Background: *BRAF* sequencing for base substituions (subs) is a standard of care for the management of patients with metastatic melanoma (MM), but comprehensive genomic profiling (CGP) to search for other types of *BRAF* genomic alterations (GA) in the disease has been limited to the research setting. In the following study, *BRAF* status was determined using a CGP assay to learn whether distinct clinicopathologic features and clinical management opportunities were associated with the detection of *BRAF* fusion GA.
Design: DNA was extracted from 40 microns of FFPE sections from 494 clinically advanced metastatic MM. CGP was performed on hybridization-captured, adaptor ligation based libraries to a mean coverage depth of >600X for 3,769 exons of 236 cancer-related genes plus 47 introns from 19 genes frequently rearranged in cancer. The results were evaluated for all classes of GA including substitutions, indels, copy number changes and gene fusions. Clinically relevant GA (CRGA) were defined as GA linked to drugs on the market or under evaluation in mechanism driven clinical trials.
Results: 158/494 (32%) of MM had *BRAF* GA. 148/494 (30%) had *BRAF* subs and indels and 4 (1%) had *BRAF* copy number changes. 8/494 (2%) MM had *BRAF* gene fusions with a wide variety of intronic partners. CGP of tumor sequences indicated that 8/8 (100%) of the *BRAF* fusions in these MM cases activated RAF kinase function. No *BRAF* fusion positive MM patients had received BRAF targeted therapy prior to CGP. For 3/8 (38%) of MM with *BRAF* fusions, the fusion was the only *BRAF* GA. 7/8 (88%) of MM with *BRAF* fusions displayed the classic spindle cell and/or epithelioid cell phenotype characteristic of Spitzoid MM. 1 (13%) of *BRAF* fusion positive MM exhibited non-Spitzoid morphology and this cases also featured a *BRAF* V600E base substitution in addition to a *BRAF* fusion. 3/7 (43%) of *BRAF* fusion positive Spitzoid MM had a *P16* (*CDKN2A/B*) partial deletion. Positive patient responses of *BRAF* fusion positive MM to anti-BRAF kinase targeted therapy will be presented. Finally, the enrichment of *BRAF* fusions in Spitzoid MM vs non-Spitzoid MM was significant (7/24 vs. 1/488, P<0.0001).
Conclusions: *BRAF* gene fusions are an exceedingly rare event in MM, are not detectable by routine hot-spot BRAF sequencing assays, but are significantly enriched in and characteristic of the Spitzoid MM histologic subtype. Given the recent evidence that *BRAF* fusions, similar to the *BRAF* V600E base substitution, can respond to anti-BRAF targeted therapy, further study of *BRAF* fusions in MM appears warranted.

474 Recurrent TERT Gene Promoter Mutations May Predict Aggressive Tumor Behavior and Common Among Melanomas From Sun Damaged Skin

Arivarasan Karunamurthy, Terry Kho, Uma Rao, Marina Nikiforova. University of Pittsburgh Medical Center, Pittsburgh, PA; University of Pittsburgh School of Medicine, Pittsburgh, PA.
Background: Human Telomerase Reverse Transcriptase (*hTERT*) gene promoter mutations are recently reported in aggressive variants of few human cancers. Cutaneous Melanomas often harbor activating *BRAF* or *NRAS* mutations and *TERT* gene mutations amplify the impact of these activating mutations by generating additional binding motifs for transcription factors. We studied the prevalence of *TERT* gene mutations in *BRAF* and *NRAS* mutated melanomas and its role in predicting biological aggressiveness of melanoma.
Design: 31 primary cutaneous melanomas (PCM) and 42 metastatic melanomas (MM) that were either *BRAF* or *NRAS* mutated were evaluated. *TERT* mutation status was determined by conventional Sanger sequencing and analyzed with the available clinco-pathological data.
Results: 39 (17PCM, 22MM) carried *BRAF* mutations and 34 carried *NRAS* mutations (14 PCM, 20 MM). *TERT* mutations were identified in 56 of 73 melanomas (77%) occuring at two common hot-spots in the promoter region (C228T and C250T) with higher frequency among PCM (84%) than MM (72%). Among PCMs with available data, *TERT* mutation was frequently observed in melanomas with higher TNM "T" stage (≤ T2:62% vs ≥ T3:87%), ulceration (absent: 60% vs present: 93%) and high mitotic index (£2/mm^2: 71% vs >2/mm^2: 100%). *TERT* mutations were detected less frequently with *NRAS* positive MM (60%) and equally among *NRAS* mutated PCM (85%), *BRAF* mutated MM (82%) and *BRAF* mutated PCM (82%). 14 of 15 (PCM + MM) melanomas (93%) arising from chronic sun damaged skin (CSDS: head, neck, scalp, forearm, hand) showed *TERT* mutations and similarly all melanomas (8 cases; 100%) with *BRAF* V600K mutation harbored *TERT* mutation. 7 PCMs from CSDS with negative sentinel lymph node status showed *TERT* mutation and 5 (70%) of them had follow up metastatic lesions.
Conclusions: *TERT* mutations are highly prevalent in both PCM and MM with UV-signature C>T substitution mutations recurring at the same hot spot promoter regions. Among primary cutaneous melanomas, *TERT* mutations are frequently associated with aggressive tumor phenotype such as increased Breslow thickness, ulceration and higher mitotic index. *TERT* mutations are also very common among melanomas arising from CSDS and with concurrent *BRAF* V600K mutations, another signature mutation of chronic sun damage.

475 mTOR, PDGFR, and C-Kit Signaling Pathway Activation in Kaposi Sarcoma

Darcy Kerr, Satya Busarla, Aliyah Sohani, Rosalynn Nazarian. Massachusetts General Hospital, Boston, MA; Aga Khan Hospital, Kisumu, Kenya.
Background: Kaposi sarcoma (KS) is an intermediate-grade vascular neoplasm with no known cure that is locally progressive with high recurrence rates and potential for wide dissemination. Current therapeutic options for advanced disease are limited by low efficacy and high toxicity. Activation of mTOR, PDGFR, and c-*kit* signaling pathway molecules has been implicated in the pathogenesis of KS and may suggest a therapeutic role for targeted inhibitors.
Design: Cases of KS were retrospectively retrieved; 276 samples contained sufficient tissue for analysis. The majority (90%) of cases were associated with HIV. Tissue microarray master blocks were created and stained with H&E, HHV8, Fli-1, CD117 (c-kit), PDGFR, and pS6 with controls. Both the intensity (0, negative; 1, weak; 2, moderate; 3, strong) and extent (0, <5%; 1, 5-25%; 2, 26-50%; 3, 51-75%; 4, >75%) of staining were scored. Multiplying scores for intensity and extent yielded total staining scores (range = 0-12) that were subdivided as follows: negative: 0, weak: 1–3, strong: ≥4.
Results: In total, 87% of the KS samples were HHV8+ (score: ³1) with 64% showing strong staining. Virtually all were Fli-1+ (95.7%) with 89% staining strongly, and pS6+ with 67.3% staining strongly (Table 1). Most were also CD117+ (92.5%) and PDGFR+ (88.1%). There was no significant difference in total staining scores for CD117, PDGFR or pS6 among patients with low (<500 cells/mm^3) or preserved CD4 counts.

	HHV8	Fli-1	CD117	pS6	PDGFR
Evaluable cores (n)	253	254	252	255	252
Total positive % (n)	87.0% (220)	95.7% (243)	92.5% (233)	96.1% (245)	88.1% (222)
Strong	64.0% (162)	89.0% (226)	48.4% (122)	67.3% (172)	42.9% (108)
Weak	22.9% (58)	6.7% (17)	44.0% (111)	28.7% (73)	45.2% (114)
Negative	13.0% (33)	4.3% (11)	7.5% (19)	3.9% (10)	11.9% (30)

Table 1: Summary of immunohistochemical findings in Kaposi sarcoma cases

Conclusions: Immunohistochemistry confirms upregulation of components of the mTOR, PDGFR, and *c-kit* signaling pathways in a large cohort of human KS samples. Of the proteins tested, overexpression of pS6, downstream of mTOR, demonstrated the highest proportion of positivity with most tumors staining strongly. These results warrant further study and support the possibility of using targeted pathway inhibitors in managing KS. Overexpression was independent of CD4 cell count, suggesting that even patients with low CD4 counts may potentially be considered as targeted therapy candidates.

476 Cutaneous Manifestations in Inflammatory Bowel Disease: A Single Institutional Study of 209 Patients

Jennifer Ko, Georgina Umberti, Deepa Patil, Karl Napekoski, Steven Billings. Cleveland Clinic, Cleveland, OH.
Background: Skin is most commonly affected by extraintestinal manifestations of inflammatory bowel disease (IBD). Cutaneous manifestations have previously been grouped into those sharing histologic features with IBD; those sharing pathophysiologic mechanisms with IBD; IBD-specific diseases; and therapy-related manifestations. Of these, erythema nodosum (EN), pyoderma gangrenosum (PG) and Sweet syndrome (SS) reportedly occur most frequently. However, a systematic analysis of the spectrum of cutaneous lesions in a controlled setting has not been performed.
Design: 4147 IBD resections classified into Crohn disease (CD) or ulcerative colitis (UC) from 2000-2013 were cross referenced with skin biopsies to investigate associated non-neoplastic skin disease and differences between CD and UC.

Results: Of 4147 patients, 209 (5%) (168 CD [80%]; 41 UC [20%]) had a total of 403 skin biopsies (1.93/patient; 89 (43%) males and 120 (57%) females). 133 biopsies (106 CD; 27 UC) were performed for non-neoplastic conditions. Granulomatous dermatitis was roughly equally present in CD (18/106;17%) (7/18 perianal location), and UC (4/27;15%) (2/4 perianal), but had an increased propensity to occur distantly in CD. Neutrophilic dermatosis (ND) was present in 35/106 CD (22%), including abscess (22), leukocytoclastic vasculitis (LCV) (5), suppurative follicultis (SF)(4), and SS (4). All cases of LCV occurred in CD. In UC, ND were more common, present in 11/27(41%), including ulcer (6), SF (3), and SS (2), making ND almost twice as prevalent in UC. Psoriasis vulgaris was more strongly represented in UC (2/27;7.4%) compared to CD (1/106; 0.9%) also. Dermal hypersensitivity reactions were more common in UC (3/27;11%) versus CD (3/106;2.8%). The contribution of medications to account for this is currently under investigation. Spongiotic/psoriasiform dermatitis was more common in CD (25/106;24%) than UC (2/27;7.4%), further broadening the spectrum of findings in CD. EN and PG rarely occurred in CD (2/106;1.8% and 1/106; 0.9% respectively), and were not seen in UC.

Conclusions: Our data suggest that there is a wide spectrum of cutaneous manifestations in IBD. While the majority has similar types of inflammatory processes to IBD (e.g. granulomatous and neutrophilic), they vary in presentation and there is a wider spectrum than appreciated. Diseases classically considered associated with IBD such as EN are relatively rare. This information should encourage dermatopathologists and dermatologists to include cutaneous manifestation of IBD in the differential of otherwise unexplained skin lesions in this population.

477 Histologic Pattern of Merkel Cell Carcinoma Sentinel Lymph Node Metastasis and Patient Immune Status Significantly Impacts Disease Outcome

Jennifer Ko, Victor Prieto, Ricardo Vilain, Richard Scolyer, Jordan Reynolds, Steven Billings. Cleveland Clinic, Cleveland, OH; University of Texas MD Anderson Cancer Center, Houston, TX; Prince Alfred Hospital, Sydney, Australia.

Background: Sentinel lymph node biopsies (SLNBx) are used to stage Merkel cell carcinoma (MCC), but the predictive value has been questioned. This reflects a lack of consensus criteria on defining meaningful positivity. For comparison, isolated immunohistochemically (IHC) positive tumor cells reportedly have no prognostic impact in breast carcinoma, while isolated melanoma cells are considered significant.

Design: 58 MCCs involving SLNBx and corresponding immunostains were reviewed for pattern of metastasis. SLNBx involvement was categorized into 5 patterns: 1-sheet-like, 2-parafollicular, 3-sinusoidal, 4-perivascular hilar, and 5-single scattered cells, and by whether tumor was identified by IHC only. Follow-up and clinical data on immunosuppression was obtained and statistically compared to pattern of metastasis (Fisher's exact test).

Results: Pattern 1, sheet-like, was found in 34 of 58 (58.6%) cases. Pattern 3, sinusoidal, was found in 11 of 58 (18.9%). Pattern 5, rare scattered cells, was found in 9 of 58 (15.5%). Patterns 2 and 4 were rare, found in 3/58 (5.2%) and 1/58 (1.7%), respectively. Pattern 1 was identifiable on H&E stains alone in all cases. Pattern 5 was only identified by IHC in all cases. Patterns 2-4 variably required IHC for diagnosis or confirmation. Follow-up currently available on 33/58 of cases showed 10/33 (30%) died of disease (DOD), 3/33 (9%) had locoregional and distant recurrence but survived, and 20/33 (61%) were alive with no evidence of disease (ANED) (mean follow-up = 48.7 months, range= 1-160). Mean time to recurrence was 9.2 months (range 2-16), and mean time to death DOD was 15 months (range=4-34). Pattern 1 compared with patterns 2-5 was associated with DOD (p=0.04). Pattern 5 was not associated with DOD (p=0.59). Immunosuppression was overrepresented in the cohort, especially those DOD. 9/31 with available data on both immune suppression and mortality were immunosuppressed (2 organ transplants, 3 hematolymphoid malignancies, 4 autoimmune diseases on steroids); 6/9 had recurrence, and 5/9 DOD. Immunosuppression was associated with DOD (p=0.05). 3/31 recurred after treatment, but are currently ANED (65-160 months), one which was transiently immunosuppressed.

Conclusions: MCC may follow the model of breast carcinoma in terms of risk stratification with SNLBx: isolated tumor cells may not be prognostically significant. Immunosuppressed MCC patients have a higher risk of DOD and may require more aggressive management.

478 Loss of Primary Cilia Distinguish Spitzoid Melanoma From Spitz Nevi and Atypical Spitz Tumors

Ursula Lang, Christine Cheung, Eszter Vladar, Jinah Kim. University of California, San Francisco, CA; Stanford University, Stanford, CA.

Background: Primary cilium are ubiquitous sensory organelles that have essential functions in cellular proliferation, differentiation, and development through recruitment and organization of a growing list of signaling molecules. The relationship between primary cilia and cancer development is an active area of investigation, and cilia loss has been described in many solid tumor types. Recently, it has been shown that benign melanocytes are ciliated, whereas the vast majority of in situ and invasive melanomas examined demonstrate significantly reduced ciliation. Due to the histopathological challenge in differentiating benign Spitz nevi, atypical Spitz tumors and spitzoid melanoma, we sought to measure the extent of ciliation in these melanocytic proliferations.

Design: We collected 16 cases, including, Spitz nevi (n=5), atypical Spitz tumors (n=6) and spitzoid melanoma (n=5), and subjected the formalin-fixed paraffin-embedded sections to immunofluorescence microscopy. Melanocytes were highlighted with Sox10 antibody and costained with antibodies to identify the basal body with gamma-tubulin and cilium with acetylated alpha-tubulin. The ratio of melanocytes expressing cilia was measured under high-power fluorescence microscopy.

Results: Based on these calculations, we demonstated that melanocytes in Spitz nevi had an average ciliation of 26% (SD: 14%); atypical Spitz tumors had 29% ciliation (SD: 14%) and spitzoid melanoma had 4% ciliation (SD: 2%). There was a significant and statistically significant decrease in number of cilia from benign Spitz and atypical Spitz to spitzoid melanoma (p=0.003).

Conclusions: These data show that primary cilia are preferentially diminished in spitzoid melanoma when compared to benign and atypical Spitz nevi. Additional studies are needed to correlate these findings with clinical outcomes and to address the role primary cilia may have in the underlying biology. The extent of ciliation may be a novel means of distinguishing benign and atypical Spitz nevi from spitzoid melanoma.

479 PD-L1 Expression in Mucosal Malignant Melanoma: Implications for PD-1/PD-L1 Blockade Therapy

Mohammed Lilo, Sneha Berry, Haiying Xu, Aleksandra Ogurtsova, Evan Lipson, Janis Taube. Johns Hopkins University School of Medicine, Baltimore, MD.

Background: Mucosal malignant melanoma (MMM) is an aggressive, rare neoplasm that lacks effective systemic therapies. The anatomic locations and absence of specific, early diagnostic signs often contribute to an advanced stage at the time of diagnosis. Agents blocking PD-1 and PD-L1 have shown anti-tumor activity in patients with advanced solid malignancies, including melanoma. Response rates have been linked to PD-L1 expression in the tumor microenvironment, with PD-L1 (+) melanomas significantly more likely to demonstrate an objective response when compared PD-L1(-) melanomas. Our previous studies indicate that one major mechanism driving melanoma tumor cell PD-L1 expression is an association with tumor infiltrating lymphocytes (TIL), consistent with an endogenous anti-tumor immune response primed for activation by anti-PD-1/PD-L1 therapies. To investigate whether MMM might be a target for PD-1/PD-L1 blockade, we analyzed PD-L1 expression in primary, recurrent, and metastatic cases of MMM as well as the association of PD-L1 expression with TIL.

Design: Seventeen formalin-fixed paraffin-embedded tumor specimens from 9 patients were identified (9 primary lesions, 3 local recurrences and 5 distant metastases) and assessed for PD-L1 expression. Immunohistochemistry was performed using the mAb 5H1. Cases demonstrating ≥5% PD-L1 membranous expression on melanoma tumor cells were considered positive. TIL were scored as mild, moderate, or severe in intensity.

Results: Tumoral PD-L1 expression was observed in 18% (3/17) cases of MMM, which is notably less than observed in cutaneous melanomas where approximately 40-50% of cases demonstrate PD-L1 expression. When present,PD-L1 expression was observed in association with TIL (p=0.006). Only one case demonstrated TIL without associated tumoral PD-L1 expression.For the four cases that had TIL present,all (4/4) were only mild in intensity.

Conclusions: Our findings, while preliminary, suggest that relative to other melanoma subtypes, MMM has a weak endogenous antitumor immune response, evidenced by both a paucity of TIL and a lack of PD-L1 expression on the tumor cell surface. These findings suggest that anti- PD-L1/PD-1 therapies in patients with MMM are likely to be more effective when used in combination, either synchronously or metachronously, with other therapies that induce anti-tumor immunity.

480 Yes-Associated Protein-1 (YAP-1) Expression in Melanocytic Lesions

Allen Miraflor, Zhongze Li, Rebecca O'Meara, Marc Ernstoff, Shaofeng Yan. Dartmouth-Hitchcock Medical Center, Lebanon, NH.

Background: The Hippo pathway is a highly conserved potent regulator of cell growth, division and apoptosis, first described in Drosophila studies. Yes-associated protein 1 (YAP-1) is a transcriptional activator in this pathway. YAP-1 has been implicated as an oncogene in multiple human tumors including liver, gastric, ovarian and lung cancers. In breast cancer, it is controversial whether YAP-1 acts as a tumor suppressor or oncogene. There is limited data on its role in melanoma. Although overexpression of YAP-1 in melanoma cell lines have shown increased cell invasiveness in vitro, mixed expression was observed in both benign nevi and melanoma. In this study, we examined the expression of YAP-1 by immunohistochemistry in melanocytic lesions, with evaluation of its correlation with clinicopathologic parameters and survival prognosis.

Design: Immunohistochemical staining with antibody to YAP-1 protein was used to examine an annotated tissue microarray consisting of a total of 361 tissue cores representing 116 primary melanomas, 24 melanoma metastases and 59 benign nevi. A semi-quantitative scoring method was used to determine the percentage of positive tumor cells and staining intensity. A proportional odds model was used to evaluate differential expression. Kaplan-Meier method was used to estimate disease specific survival, and both univariate and multivariate survival analyses were performed.

Results: Nuclear and cytoplasmic expression were observed in both benign nevi and melanoma, with lower nuclear (p<0.001) and higher cytoplasmic staining (p=0.048) significantly associated with melanoma compared to benign nevi. YAP-1 expression in primary melanomas did not show significant association with clinicopathological parameters. Univariate and multivariate survival analyses showed that tumor thickness, ulceration and mitotic rate were significantly associated with survival, but not for nuclear or cytoplasmic expression levels of YAP-1.

Conclusions: Nuclear and cytoplasmic expression of the YAP-1 protein were seen in the benign nevi and melanoma cases. However, negative nuclear expression and strong cytoplasmic expression were more significantly associated with melanoma. Unlike other tumors, YAP-1 is not a significant prognostic factor in melanoma. As a transcription factor, the absence of nuclear expression suggests YAP-1 may function as a potential tumor suppressor in a subset of melanoma. The underlying mechanism still has yet to be elucidated.

481 Loss of Bap1 Expression in Non-Melanoma Skin Cancer in Patients With Germline BAP1 Mutations

Mark Mochel, Adriano Piris, Vania Nose, Mai Hoang. Massachusetts General Hospital, Boston, MA.

Background: Patients with heterozygous germline mutations in BRCA1 Associated Protein (*BAP1*), a tumor suppressor gene, develop a tumor predisposition syndrome (OMIM #614327) with increased risk of uveal melanoma, cutaneous atypical and epithelioid melanocytic lesions, lung adenocarcinoma, clear cell renal cell carcinoma, and other tumors. *BAP1* germline mutations are found in 4% of patients with ocular melanoma and 1.5% of patients with a family history of cutaneous melanoma. Recently, loss of BAP1 expression was reported in 19 basal cell carcinomas (BCC) from 4 patients in 2 families with *BAP1* germline mutations (*Clin Genet* 2014 Jul 31). The BAP1 expression in squamous cell carcinoma (SCC) in this setting has not been reported.

Design: We previously documented loss of BAP1 nuclear expression in 19/19 melanocytic lesions in two patients with germline *BAP1* mutation and family history of uveal melanoma. We evaluated Bap1 expression in 7 BCC and one SCCIS from these same two individuals. These lesions were located in the head and neck region (6) and shoulder (2). 31 sporadic BCCs were included as controls.

Results: The BCCs in the patients with *BAP1* germline mutations showed typical features of BCC with 5 showing superficial and/or nodular components and 2 with infiltrative growth. All 7 BCCs exhibited loss of BAP1 nuclear staining while 30/31 (97%) sporadic BCCs exhibited positive Bap1 nuclear staining. The case of SCCIS showed loss of Bap1 expression in carcinoma cells.

Conclusions: Loss of BAP1 expression may be associated with the development of BCC and SCCIS in patients with germline *BAP1* mutations. The results suggest that non-melanoma skin cancer may be a component of the expanding category of tumors associated with this syndrome. Furthermore, screening for *BAP1* mutation, loss, and inactivation through BAP1 immunohistochemistry on non-melanoma skin cancers may provide a simple method for screening a patient suspected of harboring a germline *BAP1* mutation.

482 AURKA Mutation Commonly Found in Conjunction With BRAF V600E in Skin Melanoma

Susan Mockus, Grace Stafford, Christopher Potter, Guruprasad Ananda, Douglas Hinerfeld, Gregory Tsongalis. The Jackson Laboratory for Genomic Medicine, Farmington, CT; The Jackson Laboratory for Mammalian Genomics, Bar Harbor, ME; Geisel School of Medicine at Dartmouth and Dartmouth-Hitchcock Medical Center, Lebanon, NH.

Background: Activating BRAF mutations have been reported in 50% of skin melanomas, which are commonly treated with BRAF inhibitors. However, since resistance to BRAF inhibitors occurs within 5-7 months, combinations of therapies targeting the RAS/RAF/MEK/ERK pathway are actively under investigation. A further therapeutic option may be the interception of activated targets that cross-talk with the MAPK pathway, such as the Aurora kinases.

Design: DNA from ten skin melanoma FFPE samples was sequenced on Illumina HiSeq 2500 or MiSeq sequencers using a CLIA-certified 358-gene targeted sequencing assay. FASTQ files generated from Illumina's CASAVA software were submitted to a Clinical Genome Analytics (CGA) data analysis pipeline to perform automated read quality assessment, alignment, and variant calling. Clinical annotations for actionable variants and therapeutic interventions were curated through extensive literature searches and a comprehensive clinical report was written for each sample.

Results: The AURKA missense mutation, F31I, was found in conjunction with BRAF V600E in 7 of 10 melanoma samples and one sample had only the AURKA F31I. Literature searches identified AURKA F31I as an activating mutation capable of oncogenic transformation in mouse xenografts. Although AURKA amplification has been previously reported in skin melanomas, activating mutations in AURKA have not. Compilation of specific AURKA inhibitors include alisertib, ENMD-2076, and TAS-119, while pan-AURK kinases include ABT-348, AMG900, AT9283, BI-847325, cenisertib, danusertib, PF-03814735, and SNS-314. Interestingly, BI-847325 is a dual inhibitor of MEK 1,2 and Aurora kinases.

Conclusions: AURKA F31I is commonly found in conjunction with BRAF V600E in skin melanoma and provides an additional therapeutic target. BRAF inhibitors, in combination with AURKA inhibitors and/or MEK inhibitors, is a rationale therapeutic approach to consider for the treatment of skin melanoma carrying activating mutations in AURKA.

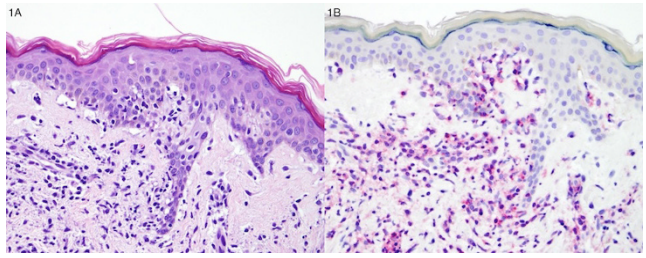
483 Epidermotropic B Cell Lymphoma

Shabnam Momtahen, Cynthia Magro. Weill Cornell Medical College, New York, NY.

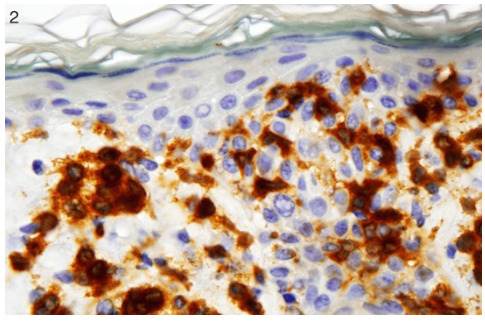
Background: Epidermotropic B cell lymphoma is a rare subset of cutaneous B cell lymphoma characterized by a diffuse skin rash affecting older adults. All of the reported cases represent marginal zone lymphoma. The exact mechanism of B cell localization to the epidermis is unclear but it might be related to certain intraepidermal cytokines and growth factors similar to the epidermotropism in mycosis fungoides (MF). Given the known CXCR3 expression in splenic marginal zone lymphoma and epidermotropic T cells of MF and its loss in nonepidermotropic tumor stage MF, our aim was to study the expression of CXCR3 in epidermotropic B cell lymphoma.

Design: The hospital database and a literature review was conducted and 7 skin biopsies (6 males, 1 female, mean age of 65 years) with epidermotropic B cell lymphoma were studied. CXCR3 expression was assessed in 5 cases using immunohistochemical techniques.

Results: Two cases were encountered in the hospital database and 5 cases had been previously reported; material was requested on the outside cases. All cases showed a pandermal infiltrate of atypical lymphocytes. The cells focally permeated the epidermis, assuming a passive pattern of colonization within the lower third of the epidermis (fig.1A). Neoplastic cells were strongly positive for CD20, CD79a and bcl-2. CD10 and bcl-6 were negative. T-cell markers highlighted scattered reactive lymphocytes. Molecular studies were performed on 4 cases and revealed B cell clonality. CXCR3-expressing epidermotropic B cells were detected in 4 of the 5 cases studied (fig.1B).



Double staining of CD79a-CXCR3 highlighted the B cell infiltrate in one case (fig.2).



Conclusions: Epidermotropic B cell lymphoma represents a subset of marginal zone lymphoma occurring more frequently in males. We speculate that aberrant expression of CXCR3 in marginal zone lymphoma of the skin is associated with migration of lymphoma cells to the epidermis and could lead to an epidermotropic pattern given the known role of CXCR3 in localization of neoplastic T cells of MF to the epidermis.

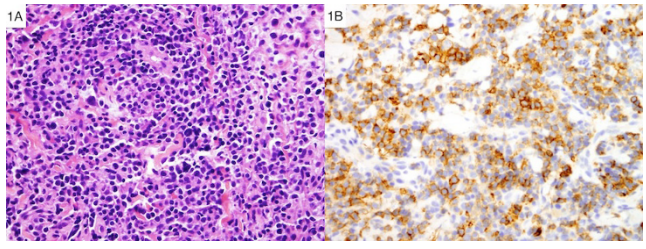
484 Primary Cutaneous Small Cell Variant of Anaplastic Large Cell Lymphoma: A Case Series

Shabnam Momtahen, Maija Kiuru, Cynthia Magro. Weill Cornell Medical College, New York, NY.

Background: Primary cutaneous anaplastic large cell lymphoma (C-ALCL) is characterized by cohesive sheets of large lymphoid cells expressing CD30. While systemic ALCL is an aggressive lymphoma, primary C-ALCL is an indolent disease. Several morphologic variants of systemic ALCL have been reported including the classic type, lymphohistiocytic and small cell variants. Small cell variant of ALCL is characterized by a prominent small cell component. Although recognized in its systemic form, it is not well characterized in the skin.

Design: A total of 10 skin biopsies from 8 patients with primary cutaneous small cell variant of ALCL were assessed using immunohistochemical stains including CD2, CD3, CD4, CD5, CD7, CD30 and ALK. T-cell receptor (TCR) gamma gene rearrangement analysis was performed using PCR.

Results: Patients were all male with a mean age of 71 years. Clinical presentation varied from a recurrent 8 cm nodule on the lower extremity to multiple persistent erythematous nodules on the face. Histopathological analysis revealed a predominant cell morphology of monomorphic appearing small to intermediate-sized lymphocytes with a minor component of larger atypical cells (figure1A). There was variable epidermotropism. In most of the cases neoplastic cells expressed CD2, CD3 and CD4 along with aberrant loss of CD5 and CD7. CD30 expression was present not only amidst the large cells but also the small and intermediate-sized atypical lymphocytes (figure1B).



In 2 cases CD4 and CD8 were both negative. ALK was performed in 4 cases and was negative. TCR gamma gene rearrangement analysis revealed monoclonality in 3 of 4 cases studied.

Conclusions: There is limited literature precedent on small cell variant of CD30 positive T cell lymphoma of the skin. Due to the unusual histomorphology, a misdiagnosis has been reported in up to 50% of the reported cases. In most of the cases CD30 expression was only encountered in the large cell component. In our series, while all tumor cells expressed CD30, a paradoxical expression of CD30 in the small cell component was encountered, a surprising finding given a tendency to associate CD30 expression with transformed and hence a large cell morphology. While ALK staining is common in systemic small cell ALCL, it is uncommon in the primary cutaneous counterpart.

485 Vemurafenib Associated Melanocytic Lesions Lack BRAFV600E Mutation and Show Preserved BAP1 Expression

Kumaran Mudaliar, Carlos Torres-Cabala, Michael Tetzlaff, Maeleine Duvic, Ana Ciurea, Sharon Hymes, Kenneth Tsai, Victor Prieto, Jonathan Curry. University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Paradoxical activation of MAPK pathway in *BRAF* wild type cells has been a proffered mechanism for vemurafenib (VEM) associated cutaneous toxicities [e.g. keratoacanthomas (KA) and squamous cell carcinoma (SCC)]. The emergence of newly appearing or changing melanocytic lesion (ML) is a recently reported VEM toxicity. IHC analysis with anti-BRAFV600E and anti-BAP1 are useful surrogate markers in evaluation of their respective mutation status. Our knowledge of mutation in ML associated with BRAF inhibitors is limited. We sought to evaluate for *BRAFV600E* and *BAP1* mutations in VEM associated ML (VEM-ML) by IHC.

Design: A retrospective review was performed in patients (pts) over a 4-year period (2010-14) in whom new or changing ML were biopsied for histopathologic examination while on VEM therapy. Also recorded were clinical information, diagnoses and presence of VEM keratinocytic alterations. There were 46 ML [37 VEM-ML and 9 control nevi (CN)]. Immunohistochemical studies (IHC) with anti-BRAFV600E (VE1) and anti-BAP1 (C4) were performed and percentage (+) cells were scored as <10%, >50%, >95%. Comparisons between the two groups were made by Chi-square and p values <0.05 were considered significant.

Results: 37 VEM-ML (23 dysplastic nevi (DN), 5 melanoma (M) [3 invasive, mean thickness = 0.5 mm, 1 in situ, 1 metastasis], 6 melanocytic nevi (MN), and 3 MN with atypia) were examined from 18 pts (M:F=3.5:1; mean age 56 y; range 19-79 y) while on therapy for treatment of advance stage melanoma (n=12) and other cancers (n=6). The average duration of VEM therapy prior to biopsy of ML was 8.4 months (m) (range 0.7-31.6 m). All new primary M in this group were associated with a nevus. 9 CN (3 DN, 6 MN) were from 8 pts (M:F=1:1.7; mean age 52 y; range 29-77 y). All 37 VEM-ML significantly lacked expression of BRAFV600E compared to 8/9 CN that were BRAFV600E positive (p <0.0001). Retained BAP1 was seen in both VEM-ML (36/37) and CN (9/9). 24/37 VEM-ML showed significantly more keratinocyte alteration (verrucous and/or follicle changes) than CN (0/9) (p<0.0071). Among the 32 nevi in the VEM-ML, 81% demonstrated moderate to severe cytologic atypia.

Conclusions: VEM-ML demonstrates preserved BAP1 and absence of BRAFV600E protein expression; thus, lack any IHC evidence of *BAP1 or BRAV600E* mutation. Within the VEM-ML, M were typically associated with a nevus and majority of MN demonstrated greater degree of cytologic atypia. The development of ML as toxicity to VEM is likely due to paradoxical activation of MAPK pathway in melanocytes that lack *BRAFV600E* mutation, similar to KA and SCC VEM toxicity.

486 Diagnostic Utility of MYC mRNA Chromogenic In Situ Hybridization in Differentiating Secondary Angiosarcomas From Atypical Vascular Lesions

Kent Newsom, Karen Fritchie, John Reith, Dongtao Fu, Wonwoo Shon. University of Florida, Gainesville, FL; Mayo Clinic, Rochester, MN.

Background: The distinction between atypical vascular lesion (AVL) and secondary angiosarcoma (AS) can be problematic. *MYC* gene amplification is present in virtually all secondary AS but not in AVL. Therefore, assessment of the MYC status is helpful in distinguishing secondary AS from its clinical and histologic mimics. In this study, we investigated the utility of a novel *MYC* mRNA chromogenic *in situ* hybridization assay as a potential ancillary tool in distinguishing secondary AS from AVL.

Design: Formalin-fixed, paraffin-embedded whole tissue sections from 13 secondary AS, 6 AVLs, 3 primary AS, 3 chronic lymphedema, and 1 ulcer site were retrieved from our surgical pathology archives. Chromogenic *in situ* hybridization for *MYC* mRNA was performed using the RNAscope® 2.0 FFPE Assay (ACD Inc. Hayward, CA). Intracellular staining was scored as "1+" (1-10 signals/cell), "2+" (10-20 signals/cell), and "3+" (³21 signals/cell).

Results: *MYC* mRNA was detected in 12/13 (92%) cases of secondary AS with 3+ staining. None of the AVL (6 cases) and primary AS (3 cases) was positive. In the cases of chronic lymphedema and ulcer site, a weak staining (1+) was observed. *MYC* mRNA expression was also occasionally observed in the overlying epidermis and hair follicular epithelium.

Conclusions: *MYC* mRNA chromogenic *in situ* hybridization is a sensitive marker for secondary AS, and positive staining in secondary AS is diffuse and strong in intensity. This novel assay may be an additional diagnostic tool in the distinction of secondary AS from AVL after radiation therapy. Although reactive endothelial cells in chronic lymphedema and ulcer site can be *MYC* mRNA positive, unlike in secondary AS, staining is focal and weak (1+). Additional studies with larger cohorts are needed to further evaluate the diagnostic utility of this novel assay.

487 Anomalous Intermediate Filament and Synaptophysin Expression in Melanoma

Ryan Romano, Jodi Carter, Andrew Folpe. Mayo Clinic, Rochester, MN.

Background: Malignant melanomas (MM) are known to express vimentin, among other intermediate filaments (IF). Though anomalous cytokeratin (CK) expression by MM has been reported, its frequency is not well-established and this phenomenon is not well-known. We have seen in consultation a number of MM with anomalous expression of CK, other intermediate filaments, or synaptophysin, and therefore studied a large group of primary and metastatic MM to determine the frequency of these events.

Design: 70 MM (22 primaries, 48 metastases) from 68 patients (48M, 20F; mean 59 years, range 17-87 years) were retrieved from our archives. Prior diagnoses were confirmed by re-review of H and E sections and relevant (e.g., S100 protein, HMB45, Melan-A, tyrosinase) immunohistochemical studies. Available sections were immunostained for CK (OSCAR and AE1/AE3 antibodies), desmin (DES), neurofilament protein (NFP), glial fibrillary acidic protein (GFAP), synaptophysin (SYN), and chromogranin A (CG). Not all cases could be tested for all markers.

Results: Cases were predominantly epithelioid (45/70, 64%) or spindle cell/desmoplastic (25/70, 36%). S100 protein, Melan-A, HMB45, and tyrosinase were positive in 57/62 (92%), 33/61 (54%), 27/57 (47%), 24/47 (51%) of cases, respectively. The 5 S100 protein-negative cases were positive for Melan-A, HMB45, and tyrosinase in 2/4 (50%), 2/3 (67%), 1/2 (50%) of cases, respectively. All cases expressed at least one melanocytic marker. Cases were positive for CK (OSCAR, 15/58, 26%; AE1/AE3, 16/40, 40%), DES (11/46, 24%), NFP (5/31, 16%), GFAP (3/32, 9%), and SYN (9/32, 28%), typically only in a minority of cells. CG was negative (0/31, 0%). Expression of more than 1 IF was seen in 17 cases (24%). All S100 protein-negative MM showed anomalous IF expression (CK- 1 case, DES- 3 cases, NFP- 1 case). Anomalous IF or SYN expression was more common in epithelioid (26/45, 58%) as compared with spindle cell/desmoplastic (9/25, 36%) MM. Overall, 50% (35/70) of cases showed anomalous expression of at least one IF or SYN.

Conclusions: Anomalous expression of all IF and SYN was found in significant subsets of MM, representing potentially serious diagnostic pitfalls. While the inclusion of consultation cases may inflate the frequency of these findings in this series, similar findings were also seen in institutional cases. MM showing anomalous IF and SYN expression may easily be mistaken for carcinomas, rhabdomyosarcomas and neuroendocrine tumors. Awareness of this phenomenon, careful histopathological evaluation, and an appropriate melanocytic immunohistochemical panel should facilitate the diagnosis of MM with unusual immunophenotypes.

488 Do Langerhans Cell Microabscesses and Eosinophils Have Diagnostic Value in Allergic Contact Dermatitis?

Gabriela Rosa, Anthony Fernandez, Alok Vij, Apra Sood, Thomas Plesec, Steven Billings. Cleveland Clinic, Cleveland, OH.

Background: Allergic contact dermatitis (ACD) is a type IV hypersensitivity reaction that manifests as a pruritic rash. Langerhans cell microabscesses (LCMAs), as well as presence of eosinophils, are considered histologic clues to the diagnosis of ACD. There have been only limited histologic analyses of ACD. We correlated presence of LCMAs and eosinophils in skin biopsies with patch test results in patients evaluated for ACD.

Design: Charts of all patients patch tested and biopsied at one institution between 2011 and 2013 were reviewed. For inclusion the biopsies had to be diagnosed as spongiotic dermatitis, psoriasiform dermatitis or mixed psoriasiform/spongiotic dermatitis. CD1a stains were performed on all cases. Two board-certified dermatopathologists and one resident physician, blinded to patch test results, independently assessed various histologic parameters, including presence of LCMAs, on routine and CD1a-stained sections, and number of eosinophils. A physician blinded to histologic analyses reviewed patch test results. Patch test results were correlated with presence of LCMAs, number of eosinophils, and other histologic parameters. Statistical analyses by Barnard's exact test were performed.

Results: 68 biopsies met study criteria. 27(40%) had ≥ 1 LCMA. 21/27 (78%) with ≥ 1LCMA were patch test positive; 6 were patch test negative (22%). Of cases with no LCMAs, 23 were patch test positive (23/41, 56%) and 18 were patch test negative (18/41, 44%). LCMAs were significantly more common in patch test positive patients (p = 0.046). Of patch test positive cases 7 had >10 eosinophils/hpf, 5 had 2-5 /hpf, and 32 had 0-1 /hpf. Of patch test negative cases 6 had >10 eosinophils/hpf, 1 had 2-5 eosinophils/hpf and 17 had 0-1 eosinophils/hpf. Eosinophil count did not significantly differ in patch test positive and negative cases, comparing >10 eosinophils vs. other (p = 0.216).

Conclusions: LCMAs are significantly more common in patch test positive cases. Though not entirely specific, the presence of LCMA suggests the possibility of allergic contact dermatitis, and we recommend mentioning this in pathology reports. There were no differences with regards to presence of eosinophils between the two groups, and absence of eosinophils in dermal infiltrates does not exclude the diagnosis of ACD.

489 INSM1: A Novel Nuclear Marker in Merkel Cell Carcinoma (Cutaneous Neuroendocrine Carcinoma)

Patrick Rush, Jason Rosenbaum, Rebecca Baus, Daniel Bennett, Ricardo Lloyd. University of Wisconsin Hospital and Clinics, Madison, WI.

Background: Merkel cell carcinoma (MCC) is a rare, clinically aggressive, cutaneous neuroendocrine (NE) neoplasm. As a tumor with small, round, blue cells, the differential diagnosis for MCC can include melanoma, metastatic small cell carcinoma, nodular hematopoietic tumors, basal cell carcinoma and atypical variants of squamous cell carcinoma. Immunohistochemistry is essential to the definitive diagnosis of MCC, and in difficult cases the diagnosis may hinge entirely on the immunoprofile of the tumor cells. INSM1 is a transcription factor expressed in tissues undergoing terminal NE differentiation. As a nuclear protein tied both to differentiation and the cell-cycle, INSM1 may offer utility over traditional, cytoplasmic markers of NE differentiation.

Design: 15 cases of MCC and 17 non-MCC specimens (including melanoma, basal cell carcinoma, squamous carcinoma, metastatic small cell NE carcinoma, and hematopoietic malignancy) were selected from tissue archives (2007 – 2014). Non-MCC specimens were selected for their potential diagnostic confusion with MCC. Cases were confirmed by H&E and were evaluated for INSM1 expression using an established protocol. INSM1 expression was evaluated for both intensity (0 – 3+) and pattern (focal, patchy, diffuse).

Results: Patients had a mean age at diagnosis of 73 (range: 61 - 89), and a male predilection of 5.5 to 1. INSM1 was detected in 14/15 MCC specimens (93%), demonstrating diffuse 2/3+ nuclear positivity. The single specimen that was negative for INSM1 was also negative for CK20, chromogranin A (CGA) and synaptophysin. Normal tissue was consistently negative for INSM1 expression, including squamous epithelium,

melanocytes, adnexae, and lymphoid tissue. Among non-MCC neoplasms, only other NE neoplasms (e.g. small cell NE carcinoma) expressed INSM1. We compared INSM1 to other markers of NE differentiation, including CGA and synaptophysin.
Conclusions: INSM1 is a sensitive marker of MCC, with strong nuclear expression. The nuclear expression pattern makes INSM1 staining easier to interpret and distinguish from background or artifactual staining. INSM1 functions in both the cell-cycle and differentiation, which may help elucidate the mechanism of disease and the associated Merkel cell polyoma virus. IHC for INSM1 is an attractive new tool in the diagnosis of MCC.

490 Merkel Cell Carcinoma – Molecular Heterogeneity, as Revealed By Integrated Genomic Analysis

Roxana Sanchez Pacheco, M Carmen Gonzalez Vela, J Pedro Vaque, Carmen Almaraz, Lorena Di Lisio, Laura Cereceda, Jose A Lopez Guerrero, Manuela Mollejo, Pablo Isidro Marron, Miguel Angel Piris. Hospital Universitario Marqués de Valdecilla, Santander, Cantabria, Spain; Instituto de Investigación Sanitaria IDIVAL, Santander, Cantabria, Spain; Biobanco, Fundación Instituto Valenciano de Oncología, Valencia, Spain; Hospital Universitario Virgen de la Salud, Toledo, Spain; Hospital Universitario Central de Asturias, Biobanco, Spain.
Background: Merkel cell carcinoma (MCC) in a highly aggressive neuroendocrine neoplasm of the skin whose molecular pathogenesis is only partially known. Polyomavirus plays a role in MCC pathogenesis, being present in a large proportion of cases. Key players in the biology of this disease such as mutated genes and activated pathways in MCC are still unveiled.
Design: Whole exome sequencing has been performed in 8 samples. A validation study has been done using immunohistochemistry in an additional group of 40 cases. We also wanted to explore a selection of signaling pathways and genetic mutations that in light of our data might relate to the MCC and hence performed immunohistochemical staining using various antibodies including β-catenin, CREB, NF-ATC1, YAP/TAZ, LEF1-L, AP-STAT3, C-MYC, p53 and p63.
Results: MCC specimens contain a highly variable number of mutated genes. RB, p53 and p63 mutations appear in a significant proportion of cases, and appear associated with a very high mutational load . Samples were also heterogeneous in the presence of polyomavirus and the expression of markers indicating RB and p53 inactivation, and activation of CREB, calcineurin/NFAT, YAP, WNT and JAK/STAT signalig pathways pathways.
Conclusions: MCC genomic/molecular analysis reveals an unexpected degree of heterogeneity. Some of the activated pathways detected could be used as markers to potentially guide individualized specific therapies.

491 Solitary (Juvenile) Xanthogranuloma: Comprehensive Immunohistochemical Characterization, Supporting True Histiocytic Lineage

Rosalind Sandell, Jodi Carter, Andrew Folpe. Mayo Clinic, Rochester, MN.
Background: Solitary (juvenile) xanthogranuloma (SXG) is an uncommon, benign lesion that usually occurs in children. The cell of origin of SXG has been the subject of debate, with hypothesized candidates including endothelium, dermal dendrocytes, dermal indeterminate cells, and the plasmacytoid monocyte, among others. We further characterized the immunophenotype of SXG with an extended immunohistochemical panel, with special attention to recently described or novel markers of histiocytic lineage, such as CD11c and CD31.
Design: Available slides and blocks from 67 cases previously coded as "SXG" were retrieved from our archives. On re-review, 28 cases either lacked sufficient tissue or were felt to represent a different diagnosis, leaving a final study population of 39 cases. These 39 cases were immunostained for Factor XIIIa, CD4, CD11c, CD163, CD31, CD45, lysozyme, and S100, using heat-induced epitope retrieval and the Ventana Autostainer. The mononuclear cells of SXG were scored as "negative," "1+" (<10% positive), "2+" (10-50% positive) and "3+" (>50% positive). Positive and negative controls were employed.
Results: The tumors consisted of a typical admixture of small, uniform, histiocytoid mononuclear cells, Touton-type giant cells, lipidized cells, scattered eosinophils and lymphocytes, and collagen bundles. Immunohistochemistry showed: Factor XIIIa 33/38 (87%), CD4 32/34 (94%), CD11c 34/35 (97%), CD163 34/34 (100%), CD31 12/29 (41%), CD45 13/30 (43%), lysozyme 22/29 (76%), and S100 0/30 (0%). Factor XIIIa was most often 3+ (53%). The five Factor XIIIa-negative cases all showed 2-3+ CD4, CD11c and CD163 expression. CD4, CD11c and CD163 essentially always showed 2-3+ staining. CD31-positive cases showed membranous, granular staining of lesser intensity than adjacent endothelial cells. CD45 expression, when present, was typically weak in intensity.
Conclusions: Our results strongly support histiocytic lineage for the mononuclear cells of SXG, with co-expression of multiple histiocyte-restricted or associated markers, including CD11c, CD4, CD163, and CD31. CD11c expression has not been previously described in SXG. Expression of CD11c and CD4 is thought to be tightly restricted to hematopoietic cells, and would not be expected in mesenchymal mimics of SXG, although study is ongoing. CD31 expression in SXG represents a potential diagnostic pitfall, as many (dermato)pathologists are unaware of CD31 expression in histiocytes, as well as endothelial cells.

492 Fatty Acid Synthase and Phospho-Acetyl-CoA Carboxylase Expression in Nodal Metastatic Malignant Melanoma and Benign Intracapsular Nodal Nevi

Maria Laureana Santos-Zabala, Travis Hollmann, Massimo Loda, Edward Stack. Memorial Sloan Kettering Cancer Center, New York, NY; BWH/DF/HCC, Boston, MA; PerkinElmer, Hopkinton, MA.
Background: Melanoma is a potentially lethal form of skin cancer for which the current standard of therapy is complete surgical removal of the primary tumor followed by possible removal of the sentinel lymph node(s). Histologic identification of metastatic melanoma in a sentinel node has significant prognostic and therapeutic implications, routinely guiding further surgical management with regional lymphadenectomy. While melanocytes in a lymph node can be identified by routine histopathologic and immunohistochemical examination, the distinction between nodal nevus cells and melanoma can be morphologically problematic. Recent studies have shown that malignant melanoma can over-express metabolic genes such as fatty acid synthase (FASN) and phosphorylated acetyl-CoA carboxylase (PACC). This immunohistochemical study aims to evaluate the expression of FASN and PACC in metastatic melanomas involving sentinel lymph nodes compared with benign nodal nevi identified in the sentinel lymph nodes of patients with cutaneous melanoma.
Design: Using antibodies against FASN and PACC, 13 sentinel lymph nodes from 13 patients with metastatic malignant melanoma and 14 lymph nodes harboring benign intracapsular nevi from 14 patients with cutaneous malignant melanoma were examined. A diagnosis of melanoma was based on cytologic atypia including histologic comparison with the primary melanoma. All nodal nevi were intracapsular (not trabecular). Expression of melanocytic markers Melan-A, S100, and HMB45 were evaluated. Percentage of melanocytes staining with FASN and PACC was scored as follows: 1-25%, 26-50%, 51-75% or >75%. Staining intensity was graded as weak, moderate or strong.
Results: All (13/13) metastatic melanomas had at least 25% of tumor cells staining for both FASN and PACC. Greater than 75% of the tumor cells stained with FAS in 7/13 cases and for PACC in 6/13 cases. Intensity of staining was variable; strong staining for FASN was observed in 69% of metastatic melanoma and PACC strongly stained 54% of cases. HMB45 showed variable expression in metastatic melanoma (6/13; 46%) and all capsular nevi were negative for FASN, PACC and HMB45 immunoreactivity.
Conclusions: All (13/13) metastatic melanoma cases involving sentinel lymph nodes were positive for FASN and PACC and no staining was observed in intracapsular or trabecular nodal nevi (0/13). These findings suggest that FASN and PACC could be utilized as valuable ancillary stains in the distinction between nodal nevi and metastatic melanoma.

493 Utility of BRAFV600E Immunohistochemistry (IHC) Expression Pattern as a Surrogate of BRAFV600 Mutation Status in 154 Patients With Advanced Stage Melanoma

Michael Tetzlaff, Penvadee Pattanaprichakul, Jennifer Wargo, Patricia Fox, Keyur Patel, Jeannelyn Estrella, Russell Broaddus, Mike Davies, Mark Routbort, Alex Lazar, Victor Prieto, Wen-Jen Hwu, Jeffery Gershenwald, Scott Woodman, Carlos Torres-Cabala, Jonathan Curry. University of Texas MD Anderson Cancer Center, Houston, TX.
Background: Successful vemurafenib targeted therapy depends on correctly identifying BRAF mutation status in a patient's melanoma. IHC analysis with monoclonal anti-BRAFV600E has emerged as a sensitive and specific surrogate of *BRAFV600E* mutation by molecular analysis, particularly when BRAFV600E IHC expression pattern in melanoma cells is strong and diffuse (D). However, the significance of focal (F) BRAFV600E IHC expression as a surrogate of mutation status is less well understood.
Design: Retrospective review was performed in melanoma patients (pt) over a 2-year period (2011-13) whose tumor specimens were tested by CLIA compliant Next-Gen-Seq (NGS) and IHC staining with anti-BRAFV600E (VE1) were performed on the same tumor source. BRAFV600E IHC and H&E sections were examined and scored (by MTT and JLC) independently of mutation status as follows: negative (neg); F (contains [-] and [+] areas); D (>95% [+]); IHC intensity=weak; moderate; strong; tumor cell phenotype [epithelioid (E) or spindle (S)]. Fisher exact test examined associations between IHC and other variables. Spearman correlation determined the agreement among IHC readers.
Results: The study included 49 primary melanomas (PM) and 105 metastases (met) from 154 pt (M:F=100:54; mean age 60 y; range: 26-87 y). NGS analysis identified 79 *BRAF* wild-type (WT) and 75 *BRAF* mutant (53 V600E, 16 V600K, and 6 nonV600E). There was excellent agreement by dermatopathologists in examination of tumor morphology, IHC expression and intensity (rho=0.99). The presence of E phenotype significantly correlated with *BRAFV600E* mutation (p=0.0085). Interpretation of BRAFV600E IHC was as follows: 98=neg; 10=F; 46=D. Tumors with IHC=F or D were significantly associated with presence of *BRAFV600E* mutation (p <0.0001). 7/10 cases with IHC=F harbored a *BRAFV600E* mutation while the remaining 3 were either WT or *nonBRAFV600E* mutation. Strong to moderate IHC intensity was significantly associated with *BRAFV600E* mutation (p<0.0001). There was some evidence of IHC=F being more common in met compared to PM samples (p=0.05). Cumulative IHC=F and D sensitivity (Se) and specificity (Sp) were 98% and 96%, respectively (resp). The PPV and NPV were 93% and 99%, resp. Se and Sp of IHC=F was 100% and 70%, resp.
Conclusions: Overall, BRAFV600E IHC demonstrates high Se and Sp in detection of *BRAFV600E* mutation. Although both IHC=F and D significantly correlated with presence of *BRAFV600E* mutation, more cautious interpretation of IHC=F is warranted and correlation with molecular study is necessary given its lower Sp.

494 Morphologically Low-Grade Spiradenocarcinoma: A Clinicopathological Analysis of 20 Cases With Emphasis on Behavior

Michiel van der Horst, Zlatko Marusic, Jason Hornick, Bostjan Luzar, Thomas Brenn. Western General Hospital and The University of Edinburgh, Edinburgh, United Kingdom; Clinical Hospital Center Sestre Milosrdnice, Zagreb, Croatia; Brigham & Women's Hospital and Harvard Medical School, Boston, MA; University of Ljubljana, Ljubljana, Slovenia.

Background: Spiradenocarcinoma is a rare malignant skin adnexal tumor, classified as low-grade, high-grade or sarcomatoid based on morphological appearances. While these tumors have traditionally been regarded as aggressive neoplasms with high metastatic and mortality rates, it has recently been proposed that at least the morphologically low-grade tumors behave in a more indolent fashion. Limited information is available, however, with only 18 such cases published so far.

Design: 20 morphologically low-grade spiradenocarcinomas were identified from departmental and referral files. H&E stained sections were reviewed, and follow-up data were obtained from hospital files and referring pathologists.

Results: The tumors were solitary with a median size of 2.8 cm (range: 0.8 to 7 cm). There was a predilection for the head and neck of elderly patients (median age: 70 years; range: 53 to 92) without significant gender bias. Histologically, the tumors were multinodular and located in deep dermis with additional involvement of subcutis. A pre-existing eccrine spiradenoma was present in all cases, and an additional cylindromatous component was noted in three tumors. The malignant aspect was characterized by loss of the characteristic dual cell population with expansile growth, up to moderate cytological atypia and increased mitotic activity (median: 6/10 HPF; range: 1 to 28). Additional findings included squamoid differentiation (n=8), ulceration (n=3) and necrosis (n=8). Follow-up (median: 67 months; range: 13 to 132) available for 16 of 20 patients (80%) revealed a local recurrence rate of 18% but no metastases or disease-related mortality.

Conclusions: In this large study with long-term follow-up, we demonstrate that spiradenocarcinomas with low-grade morphology pursue an indolent disease course characterized by occasional local recurrences. Metastases and disease-related mortality appear to be exceptionally rare, and complete local excision is the treatment of choice.

495 Squamoid Eccrine Ductal Carcinoma: A Clinicopathological Study of 18 Cases

Michiel van der Horst, Adriana Garcia Herrera, Eduardo Calonje, Thomas Brenn. Western General Hospital and The University of Edinburgh, Edinburgh, United Kingdom; St. John's Institute of Dermatology and King's College London, London, United Kingdom.

Background: Squamoid eccrine ductal carcinoma, also referred to as adenosquamous carcinoma of the skin, is a rare and only poorly documented malignant skin adnexal tumor, showing squamous as well as duct differentiation. It is currently regarded to be of low-grade malignant potential but limited follow-up information is available.

Design: 18 squamoid eccrine ductal carcinomas were identified from departmental and referral files. H&E stained sections were reviewed and immunohistochemistry for CEA and EMA was examined to confirm duct differentiation in difficult cases. Clinical follow-up data were obtained from patient records and referring pathologists.

Results: The tumors presented as nodules or plaques with a median size of 1.5 cm (range: 0.5 to 2.5 cm) and a strong predilection for the face (n=13). The extremities (n=2), neck (n=2) and chest (n=1) were less frequently involved. The patients were elderly (median age: 71 years; range: 10 to 96) with a strong male predominance of 4:1. Histologically, these poorly demarcated tumors are characterized by an infiltrative growth pattern within dermis and additional invasion of subcutis in 67%. Median tumor thickness was 5.1 mm (range 1.5 to 18 mm). In the superficial aspects the tumors differentiated towards well-differentiated squamous cell carcinoma with multifocal epidermal connection and a background of squamous cell carcinoma in situ in 2 cases. In the deeper reaches, the tumors were organized in cords and strands showing duct differentiation and a surrounding desmoplastic stroma. Cytological atypia ranged from moderate to severe and the mitotic index was increased (median: 10/10 HPF; range: 0 to 40). Additional findings were ulceration (50%), necrosis (39%), lymphovascular and perineural infiltration (both 11%). Follow-up (median: 37 months; range: 7 to 99), available for 15 patients (83%), revealed a local recurrence rate of 11%. Three patients had metastasis to locoregional lymph nodes and one patient died of metastatic disease. One patient died of unrelated and two patients died of unknown causes.

Conclusions: Our study further outlines the histological spectrum of squamoid eccrine carcinoma and emphasizes its clinical behavior with risk for local recurrence and potential for more aggressive behavior with metastasis and rare disease-related mortality. Recognition is important to differentiate it from squamous cell carcinoma and other skin adnexal carcinomas, including eccrine porocarcinoma, eccrine ductal carcinoma and microcystic adnexal carcinoma.

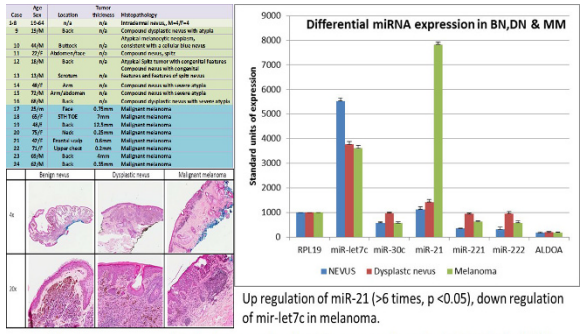
496 Utility of mir-21 Detection By Rapid Chromogenic In-Situ Hybridization (CISH) in the Differentiating Malignant Melanoma (MM), Dysplastic Nevus With Atypia (DN) & Benign Nevus (BN)

Puneeta Vasa, Ashis Mondal, Amyn Rojiani, Ravindra B Kolhe. Georgia Regents Univeristy, Augusta, GA.

Background: Differentiating MM from DN can be a diagnostic challenge due to overlapping morphology and immunophenotype. The aim of this study is to identify similarities and differences among these categories & to evaluate the utility of mir-21 detection by CISH in differentiating. Recently, a class of noncoding RNAs called miRNAs has emerged as critical gene regulators in cell growth & development. In melanoma, miRNAs have aberrant expression, with possible applications in diagnosis. Methods of sub-cellular localization of mi-RNA's are essential to understand their biological roles and their contribution to disease along with diagnosis.

Design: Archival blocks were retrieved and slides were reviewed with clinical information on 8 cases each entity. Ten 10mm sections with >50% lesion from each case were used for analysis. The miRNA expression profile was evaluated using this assay including a housekeepers and negative control on HTG genomics qNPA molecular technology platform. The ArrayPlate is then imaged via HTG's SuperCapella to measure the expression of every gene in all of the wells. Samples were run in triplicate and data was normalized to RPL19. For mir-21 CISH manufactures protocol (Exiqon miRNA ISH Optimization Kit 2 FFPE) was followed on all 24 cases.Nuclear mir-21 CISH stains were graded as: negative(-), weak (1+), moderate (2+) or strong (3+) and distribution as focal (<10%), patchy (10-50%) or diffuse.

Results: The miRNA expression profiles of MM vs DN vs BN, show significant (>6 times, p<0.05) upregulation of mir-21, and down regulation of mir-let7c in melanoma.



Up regulation of miR-21 (>6 times, p <0.05), down regulation of mir-let7c in melanoma.

Patient summary, histology and mir-21 expression in BN, DN & MM

Neoplastic cells in MM showed prominent nuclear staining for miR-21.

Conclusions: Let-7c is well documented tumor suppressor microrna located on 21q21 which shows frequent loss in metastatic tumors. Loss or reduction of let-7c leads to Ras overexpression, thus, promoting cellular growth and contributing to tumor genesis In contrast, miR-21, located on chromosome 17q23.1, is the most commonly overexpressed oncogenic miRNA & is associated with a poorer prognosis most of the tumors. In this study, we found a significant correlation between the over-expression of miR-21 and Clark levels.

497 Cutaneous Epithelioid Angiomatous Nodule: A Report of a Series With Marked Cytologic Atypia; Potential Diagnostic Pitfall

Ami Wang, Zaid Saeed Kamil, Ayman Alhabeeb, Runjan Chetty, Danny Ghazarian. University of Toronto, Toronto, ON, Canada.

Background: Cutaneous epithelioid angiomatous nodule (CEAN) is a very rare and recently recognized vascular proliferation with 54 reports in the literature to date. This benign entity could be easily confused with malignant tumors such as epithelioid hemangioendothelioma and angiosarcoma, especially when arising in immunocompromised patients. CEAN represents an important diagnostic pitfall, which could lead to significant patient anxiety and morbidity from unnecessary treatment. In all cases reported so far, the lack of cytologic atypia has been described as a reliable feature to distinguish CEAN from malignant vascular tumors. We report an unusual case of CEAN with marked cytologic atypia and brisk mitotic activity and discuss 4 other typical cases from our institution. We also present the defining clinical and pathological features that would enable pathologists to correctly classify epithelioid vascular lesions.

Design: Five cases of CEAN were retrospectively identified in the pathology database at our institution. The clinicopathological characteristics of the 5 cases were reviewed. A review of the literature was performed.

Results: Clinically, the lesions presented as well-circumscribed solitary or multiple lesions on the neck, shoulder, arm and back. Age distribution ranged from 18 to 61 years. One patient was on post-renal transplant immunosuppression therapy. Histologically, the 4 typical cases of CEAN are characterized by solid proliferation of epithelioid and spindled cells with vesicular nuclei, conspicuous nucleoli, abundant eosinophilic to clear cytoplasm and occasional intracytoplasmic vacuoles. In the unusual case, marked cytologic atypia and mitotic activity up to 16 per 10 HPF are seen. By immunohistochemistry, lesional cells are positive for vascular markers and are negative for HHV-8. In all 5 cases, there is no evidence of infiltrative margin, atypical mitoses or necrosis.

Conclusions: CEAN is a benign epithelioid vascular proliferation that can be associated with immunosuppression and shows cytologic atypia and brisk mitotic activity, features that can be mistaken for malignant vascular tumors. However, CEAN does not show other malignant features such as atypical mitoses, necrosis or infiltrative growth pattern. We emphasize relationship of the atypical features with the patient's impaired immune status in of our cases which should be explored in future studies to avoid the faulty diagnosis of more ominous lesions.

498 Prognostic Significance of Rare Intraparenchymal Immunohistochemical Positive Cells in Sentinel Lymph Nodes From Melanoma Patients

Daniel Wimmer, Amin Hedayat, Zhongze Li, Eryn Bagley, Marc Ernstoff, Dorthea Barton, Shaofeng Yan. Dartmouth-Hitchcock, Lebanon, NH.

Background: Sentinel lymph node (SLN) biopsies are useful for detecting lymph node metastases and are an important prognostic tool in melanoma. Histomorphology and immunohistochemical (IHC) studies are used to evaluate the presence of metastatic melanoma in sentinel lymph nodes. Occasionally, cases have isolated intraparenchymal IHC positive cells in SLN without corresponding cytologically atypical cells seen on

hematoxylin and eosin (H&E) stained sections. The significance of rare IHC positive cells in a lymph node is unknown. This study investigates the prognostic significance of IHC positive cells in SLN of patients with melanoma.
Design: Clinical and diagnostic records from a tertiary care center were reviewed to identify melanoma patients who received a sentinel lymph node biopsy between 2000 and 2012. Clinical outcomes and disease specific survival of patients with rare IHC positive cells in their SLN were compared to patients with negative SLN and those with SLN positive for metastatic melanoma using Kaplan-Meier analysis.
Results: Overall, 826 patients with melanoma met the study criteria. Within this group, 127 patients had metastatic disease in their SLN, 639 patients had negative SLN, and 60 had rare solitary intraparenchymal IHC positive cells in their SLN (positive for either MART-1 or HMB45, and/or S-100 protein) without corresponding atypical cells seen on H&E stained sections. The mean follow-up time for all patients was 58.8 months.
To determine the disease specific survival, control groups were standardized. All 127 patients with positive SLN were compared to 127 patients from the negative SLN group, who were selected based on age and sex. Survival data was analyzed along with those patients who had rare IHC positive cells in their SLN.
The location within lymph nodes of IHC positive cells, histomorphology, presence of capsular nevi, disease recurrence, and histopathologic features of primary lesions were also evaluated.
Disease specific survival of patients with rare IHC positive cells in SLN was not significantly different from the 127 patients with negative SLN (P=0.69), but it was statistically different from patients with SLN positive for metastatic melanoma ($P < 0.0001$).
Conclusions: This study demonstrates that rare IHC positive cells in SLN without corresponding atypical cells seen on H&E stained sections have disease specific survival comparable to patients with negative SLN. Further studies with long term follow up are needed.

499 RNA Sequence Analysis of Spitzoid Melanocytic Tumors

Gang Wu, John Easton, Seungjae Lee, Yongjin Li, James Dalton, Raymond Barnhill, Alberto Pappo, Armita Bahrami. St. Jude Children's Research Hospital, Memphis, TN; Institut Curie, Paris, France.
Background: Kinase activation by gene fusions has been recently described as a common mechanism that drives tumorigenesis in spitzoid melanocytic tumors (SMT). We performed whole-transcriptome sequencing (RNA-seq) in 5 SMT to characterize the landscape of structural variations in these lesions.
Design: RNA was extracted from formalin-fixed paraffin-embedded material in 5 SMT samples, including 4 primary tumors and 1 metastatic tumor, using the Maxwell system (Promega). RNA was quantitated by fluorescence dye, using the Quant-iT (Life Technology) RNA assay. RNA quality was evaluated by using a 2100 Bioanalyzer (Agilent Technologies) with a Nano RNA 6000 Chip. RNA-seq libraries enriched for coding regions were prepared by using the Truseq RNA Access Library Prep Kit (Illumina), following the manufacturer's protocol for RNA input quantity relative to RNA quality. The sequencing was performed on a HiSeq2000 to generate 100-bp paired-end reads. Structural variations (SV) were detected by using CICERO, a novel algorithm that uses local assembly to identify SV in RNA-seq. BAC clones (BACPAC Resources) were used to develop break-apart probes for the following genes: *BRAF* (RP11-837G3, RP11-948O19), *NTRK1* (CH17-67O18, RP11-1038N13), *ALK* (CytoCell), *PTPRZ1* (CH17-132B19, RP11-99L10), and *IL6R* (CH17-169C19, RP11-627K14).
Results: SMT occurred in 3 children (age 2–7 years) and 2 adolescents (age 13 and 14 years) and involved the lower extremities (n=3), ear (n=1), and trunk (n=1). One patient died of disseminated disease, and three patients developed large nodal metastasis. A kinase fusion was identified in each SMT, including TPM3-NTRK1 (2 tumors), BRAF-EML4 (1 tumor), BAIAP2L1-BRAF (1 tumor), and TPM3-ALK (1 tumor). All the predicted chimeric proteins were expressed at high levels and contained an intact kinase domain. In addition, we detected other fusion events in 3 SMT, including ARID1B-SNX9 (1 tumor), PTPRZ1-NFAM1 (1 tumor), IL6R-TPM3 (1 tumor), and IL6R intragenic SV (1 tumor). Gene rearrangements were confirmed by fluorescence in situ hybridization in each tumor.
Conclusions: We identified novel fusion partners for *BRAF* and several other fusion genes of unknown function in SMT. These findings demonstrate the diversity of gene fusions that define the molecular heterogeneity of these neoplasms.

500 BRAF, KIT, NRAS, GNAQ and GNA11 Mutation Analysis in Common, Cellular and Malignant Blue Nevi

Ismail Yilmaz, Mehmet Gamsizkan, Sule Sari, Cuyan Demirkesen, Aylin Heper, Banu Yaman, Aylin Calli, Gizem Narli, Zafer Kucukodaci, Ufuk Berber, Dilaver Demirel, Taner Akalin, Nesimi Buyukbabani, Murat Demiriz. GATA, Istanbul, Turkey; Military Hospital, Erzurum, Turkey; I.U.I.F.M., Istanbul, Turkey; I.U.C.F.M., Istanbul, Turkey; A.U.F.M., Ankara, Turkey; E.U.F.M., Izmir, Turkey; I.A.U.F.M., Izmir, Turkey.
Background: Malignant blue nevi (MBN) are extremely rare dermal melanocytic tumors that arise in association with cellular blue nevi (CBN), common blue nevi (BN) or de novo. The frequency of BRAF, NRAS and KIT mutations differ between histological types and locations of malignant melanomas. These mutations are rarely observed in blue nevi. Recently, activating mutations in GNAQ/GNA11 genes have also been shown in BN and uveal melanomas. Molecular studies in MBN are limited and there is no large series. The aim of the present study was to analyze the prevalence of BRAF, NRAS, KIT, GNAQ and GNA11 gene mutations and their association with clinicopathological features in MBN cases.
Design: Eighty two cases (12 MBN, 35 CBN, 35 BN) from 7 different institution between 1996 and 2014 were included in our study. The diagnosis of MBN cases were confirmed in a meeting by the participation of all institutions. For mutation analysis, DNAs were isolated from manually microdissected unstained histological sections. Exon (ex.) 15 of BRAF, ex.9/11/13/17/18 of KIT, ex.2/3 of NRAS, ex.4/5 of GNAQ and GNA11 genes were amplified by PCR and the amplicons were submitted to direct sequencing in both directions by using Big Dye Terminator kit and analyzed in the ABI 3730 automatic sequencer.
Results: The female/male ratio of patients was 42/40, with a mean age of 32.7±19.7 years. GNAQ/GNA11 ex.5 mutation was the most frequent mutation along all cases (%48.8), with a ratio of 37.1% (13/35) in BN, 65.7% (23/35) in CBN and 33.3% (4/12) in MBN. BRAF V600E mutation was detected in 3 of 12 (%25) MBN cases while none of the CBN and BN cases harbored BRAF mutation. Two of BRAF mutant cases were located in scalp, one was located at toe and one of them had lymph node metastasis. GNA11-Q209L mutation was detected in 2 MBN cases. None of the cases harbored NRAS ex.2/3, KIT ex.9/11/13/17/18 and GNAQ/GNA11 ex.4 mutations.
Conclusions: GNAQ/GNA11 mutations are detected commonly in MBN, CBN and BN with the highest ratio in CBN group. It is a striking finding that BRAF V600E mutation was detected in 3 of MBN cases whereas we observed none in BN and CBN cases. Our findings support the suggestion that blue nevi and variants represent a different melanocytic neoplasia. We think that our study will contribute to the literature that has limited data about the molecular alterations in dermal melanocytic lesions.

Education

501 Evaluation of In-Service Training of Residents and Fellows for Fine Needle Aspiration (FNA) Biopsy on Phantom Lesions

Amarpreet Bhalla, Linette Mejias-Badillo, Yasin Ahmed, Daniel Neill, Michael Kruger, Dilip Batra, Vinod Shidham. Wayne State University, Detroit, MI; Quest Labs, Orlando, FL.
Background: FNA biopsy training on Phantom Lesion (PL) for Pathology residents and Cytopathology fellows at the beginning of the academic session was introduced at our institution in July 2010. It includes a didactic on the technique of FNA, followed by demonstration on the PL prepared from banana piece embedded in caulk (http://www.jove.com/video/1404). Thereafter, the trainees practice FNA proficiency with smear preparation from aspirates. We evaluated the requirement and utility of training, level of comfort in transitioning to actual patients, and its role as a career enhancement tool.
Design: A survey based on Kirkpatrick's training evaluation model was designed on Surveymonkey.com for 75 participants (present and past trainees from our program). A total of 45 responded. They were divided into 2 groups (Gp): Gp A- 14 trainees prior to introduction of FNA training and Gp B- 31 trainees who underwent the training. These anonymous responses were statistically analyzed.
Results: Training evaluation was scored on a summative scale by enumeration of responses and descriptive statistics for rank measures.

Kirkpatrick training level	Training level objective	Min score	Maxscore	Mean score
1	Perception of training	6	10	8.58
2	Learning by getting acquainted and practicing	3	9	5.29
3	Transfer by transitioning to patients	4	10	7.74
4a	Results by overall learning assesment	6	17	11.58
4b	Results by evaluation of role as career enhancement tool	1	6	3.23

The utility of training in performing FNA was measured on a scale of 1-5 and scored a collective mean of 4.2. The difference in perception of requirement of training between Gp A and Gp B was not statistically significant (p 0.421). Assessment of confidence level of performing FNA as a trainee was evaluated by Mann-Whitney U test with comparison of mean rank scores. Respondents with training scored 30.0 and without training scored 7.5 with statistically significant difference (p 0.001).
Conclusions: The training was perceived as excellent (score 8.58) on Kirkpatrick level 1. The learning and transfer components were above average with a potential for improvement. The utility and requirement of FNA training was considered high by all. They were neutral about the role of training as a career enhancement tool. The trainees in Gp B conveyed higher confidence level than those in Gp A.

502 Rapid Access and Dissemination of Pathology Knowledge Using an Open Access Wiki

Michael Bonert, Adnan Karavelic, Angela Tate, Yuan Gao, Sarah Barksdale, William Dubinski, Stephen Raab. University of Calgary, Calgary, Canada; Memorial University of Newfoundland, St. John's, Canada; Sullivan Nicolaides Pathology, Brisbane, Australia; Humber River Hospital, Toronto, Canada.
Background: Pathology information sources, whether electronic or paper based, are largely commercial, often difficult to search and rarely link to one another. They also infrequent use content management software to track revisions that facilitate an open and rapid review, allow discussion among editors or solicit input from readers.
Design: We assessed the usability, usage and development of a wiki-based, secondary source of free pathology information that leverages media, software and organizational elements from Wikipedia. The site was accessible to staff & residents at one health authority (trial site) for a year and a seminar on editing was held. Subsequently, the site was launched, usage data collected using the software 'awstats', and automated editing trialed to sort and change the virtual pathology cases on the main page.
Results: The site contained 1260 diagnoses, 1360 images, 5650 pages, a search function, summary boxes and page navigation boxes. The integrated case simulator was useful for self learning, including assessment of uncommon diagnoses, guiding choice of