515 The Value of Smoking, Nodule Number and Known Extrapulmonary Adenocarcinoma in Distinguishing Primary Lung Adenocarcinoma from Metastatic Adenocarcinoma

B Zhu, S Dalal, D DeFrias, X Lin. University of Illinois at Chicago, Chicago, IL; Northwestern University, Chicago, IL.

Background: Lung cancer is one of the most common cancer and the leading cause of death world-wide. The lung is also the organ that is most frequently involved by metastatic adenocarcinoma (MA). It is important to distinguish primary lung adenocarcinoma (PLA) from MA to optimize therapy. We assess the value of clinical information (smoking, nodule number and known extrapulmonary adenocarcinoma (EPA) in differentiating PLA from MA.

Design: 204 cases with lung nodules diagnosed as adenocarcinoma by FNA and/or needle core biopsy were retrieved. The prior history of EPA, smoking and nodule number detected by CT scan was also retrieved. Based on morphology, IHC results and surgical resection, the patients were divided into 3 groups, PLA-EPA, PLA-without EPA (PLA-WOEPA) and MA.

Results: See Table 1 and Table 2.

Correlation of PLA with smoking and multiple nodules

Group	No.	Smoker (%)	Multiple Nodules (%)
PLA-EPA	55	37 (37.3)	30 (54.5)
PLA-WOEPA	88	71 (80.7)	48 (54.5)
MA	61	24 (39.3)	52 (85.2)
P1		< 0.005	< 0.005
P2		< 0.005	< 0.005

Chi-square test. P1: Comparison of PLA-EPA with MA: P2: Comparison of PLA-WOEPA with MA.

Bayesian probabilities of PLA in patientssmoking, multiple nodules and EPA

Category	No. (Total 143)	Prior Probability (%)	Posterior Probability (%)
Smoker	108	75.5	87.8
Non-smoker	35	24.5	43.2
Multiple Nodules	78	54.5	73.7
Single Nodule	65	45.5	66.2
EPA	55	38.5	59.5
WOEPA	88	61.5	78.9

Conclusions: 1. The incidence of smoking in PLA was much higher than MA, and smoker had a higher posterior probability to be PLA than a non-smoker, indicating that smoking plays a significant role in PLA no matter with or without a history of EPA.

2. The incidence of smoking in PLA-EPA was less than PLA-WOEPA, suggesting that besides smoking, other factors, such as occupational exposure, hereditary factors, genomic mutations of tumor suppressor genes, etc., also play a role in the increased incidence of PLA in the patients with a history of EPA.

3. The incidence of multiple nodules in PLA was less than MA. However, a patient with multiple nodules had a similar posterior probability to be PLA to a single nodule.

4. A patient with EPA had a lower posterior probability to be PLA than WOEPA.

5. Although our data suggested that a patient with smoking, single lung nodule or WOEPA had higher chance to be PLA, all these clinical information are not reliable and helpfull in differentiating PLA from MA as the incidence of non-smoking history, multiple nodules and presence of EPA is still high in PLA, 24.5%, 45.5% and 38.5%, respectively.

Dermatopathology

516 Monocytic Leukemoid Papulosis: A Self Limiting Cutaneous Proliferation Associated with Clonal Myeloproliferative Disorders

RM Abraham, S Verma, CS Friedman, W Tam, NM Drucker, CM Magro. Weill Cornell Medical College, New York, NY; Memorial Sloan-Kettering Cancer Center, New York, NY; Yale University, New Haven, CT.

Background: We propose a new clinical entity, monocytoid leukemoid papulosis (MLP), characterized clinically by self-remitting papules akin to lymphomatoid papulosis (LyP), a cutaneous, self-remitting lymphoproliferative disorder. Morphologically and immunophenotypically, MLP can resemble blastic plasmacytoid dendritic cell neoplasm (BPDCN), a highly aggressive neoplasm which primarily presents in the skin, due to histopathologic and immunophenotypic overlap. However, in contrast to BPDCN, the cells of interest in MLP express myelomonocytic markers and are monocytic morphologically, conferring a myeloid dendritic cell (MDC) phenotype. MLP is associated with chronic myeloproliferative disorders (MPD).

Design: 9 cases of MLP were prospectively encountered in the routine and referral practices of one author (CMM). Their light microscopic appearances were correlated with immunohistochemistry and clinical features.

Results: Patients ranged from 64 to 84 years of age with 6 males and 3 females. All patients had a waxing and waning papular skin rash. Four patients had underlying chronic myelomonocytic leukemia (CMML), 1 with myelodysplastic syndrome, 2 with myelofibrosis and 2 patients with no history of MPD. The clinical courses were variable; 1 patient died of transformation to acute myeloid leukemia and one patient experienced CMML relapse. The other 7 patients are alive and stable. At least 6 of the patients had a peripheral blood monocytosis. In each case, biopsies showed a well differentiated monocytoid infiltrate with a distinctive immunophenotype overlapping with BPDCN with variable CD4, MXA, CD56, TCL1 and CD123 positivity. However, in contrast to BPDCN, the cells expressed the myeloid markers CD68, CD11c, and lysozyme which are characteristically negative in BPDCN. The cases also variable positivity for the terminally differentiated dendritic markers CD14, CD83, HLADR and BDCA-3.

Conclusions: MLP presents as an indolent, papular, cutaneous infiltrate of mature monocytes with myeloid dendritic cell differentiation. They are seen in the setting of underlying chronic myeloproliferative disorders and are associated with peripheral blood monocytosis. There is no definitive association between MLP and a more aggressive clinical course at least in the majority of cases studied in this series. MLP should not be confused with BPDCN or with acute myeloid leukemia cutis, two far more aggressive conditions.

517 Borderline Deep Penetriating Nevi: A Unique Subset of Ambiguous Melanocytic Tumors with Malignant Potential and Normal Cytogenetics

RM Abraham, R Guo, S Li, X Wang, S Proper, M Mihm, AN Crowson, CM Magro. Weill Cornell Medical College, New York, NY; Memorial Sloan-Kettering Cancer Center, New York, NY; University of Oklahoma Health Sciences Center, Oklahoma City, OK; Brigham and Women's Hospital and Harvard Medical School, Boston, MA; Regional Medical Laboratories, Tulsa, OK; Center for Dermatology and Skin Surgery, Tampa, FL.

Background: Deep penetrating nevi (DPN) are a relatively uncommon subtype of melanocytic nevi with characteristic morphologic features. A small subset of these lesions exhibit atypical features (cytologic and architectural atypia, mitotic activity) seen in melanoma that create a histopathologically and biologically ambiguous category. These nevi we term the DPN variant of borderline melanocytic tumor (borderline DPN). Unequivocal melanomas can show morphologic features of DPN, which have been termed plexiform melanomas. There is little literature on borderline DPN and whether these lesions progress to overt clinical melanoma with fatal outcomes.

Design: 40 cases of borderline DPN lesions were identified along with 6 cases of plexiform melanoma. Clinical follow up was obtained, along with cytogenetic analysis in the form of fluorescent in situ hybridization (FISH) and/or comparative genomic hybridization (CGH).

Results: The borderline DPN cases included 24 females and 16 males (mean age of 36 years). Common sites included the face and arm. Of sentinel lymph node biopsies performed, 1/3 of cases showed lymph node involvement. All patients in whom a more aggressive clinical approach was adopted remain free of disease. All 7 borderline DPN cases tested by CGH showed normal cytogenetics as did 7 of 9 cases tested by FISH. Of the plexiform melanomas, 4/6 patients died of disease. In 3 cases there was morphologic progression from a borderline DPN to overt melanoma. In one case of progression, cytogenetics was normal in the borderline DPN and then abnormal in the progressed melanoma; the other two fatal cases of progressive disease had normal cytogenetics.

Conclusions: Borderline DPNs are a unique subset of ambiguous melanocytic tumors associated with a high incidence of regional lymph node disease and exhibiting the potential for melanoma progression despite a normal cytogenetic profile. Patients with these lesions should be aggressively managed, with at least complete re-excision and consideration of sentinel node biopsy, regardless of cytogenetic data.

518 Comparison of Immunohistochemistry and Polymerase Chain Reaction in Detection of BRAF p.V600E Mutations in Melanomas

A Al Habeeb, R Jin, S Kamel-Reid, E Torlakovic, C Wei, D Ghazarian. University Health Network, Toronto, ON, Canada.

Background: As the major type of BRAF point mutation, BRAF p.V600E mutation recently emerged as a critical biomarker greatly utilized in the treatment of metastatic melanoma, which is most commonly assessed with an allele-specific polymerase chain reaction (PCR) methodology. A novel method in detecting BRAF mutation by immunohistochemical staining was developed recently with a BRAF pV600E mutation–specific monoclonal antibody (named VE1 antibody).

Design: This study compares the detection of BRAF p.V600E mutation between PCR and immunohistochemistry using the VE1 antibody. A total of 61 tissue samples from melanoma patients were evaluated by these two methods.

Results: PCR analyses revealed 32 positive, 26 negative and 3 inconclusive cases in the BRAF p.V600E/K mutation detection. Immunohistochemistry identified 91% and 88% of positive and negative cases respectively compared to that from PCR results. Further, 3 inconclusive cases in PCR detection were identified as BRAF p.V600E mutation positive by immunohistochemical staining.

Conclusions: In conclusion, the correlation rate between these two methods is excellent (89.6%) and immunohistochemistry can play an important role in detecting BRAF pV600E mutation, especially in PCR inconclusive cases.

519 Evaluation of Advanced Stage Squamous Cell Carcinoma of the Skin by Next Generation Sequencing Opens the Door for New Routes to Targeted Therapies

RN Al-Rohil, AJ Tarasen, K Wang, CE Sheehan, GA Otto, G Palmer, R Yelenksy, D Lipson, G Palmer, SM Ali, D Morosini, VA Miller, PJ Stephens, JS Ross. Albany Medical College, Albany, NY; Foundation Medicine Inc, Cambridge, MA.

Background: Although skin squamous cell carcinomas (SSCC) are rarely high stage, on occasion they can be life threatening both due to local extension or metastasis. We hypothesized that comprehensive genomic profiling of high stage SSCC could identify genomic-derived drug targets of therapy for patients with conventional therapy-resistant disease.

Design: Hybridization capture of 3,769 exons from 236 cancer-related genes and 47 introns of 19 genes commonly rearranged in cancer was applied to≥ 50ng of DNA extracted from 25 SSCC FFPE specimens and sequenced to high, uniform coverage. Genomic alterations (base substitutions, small indels, rearrangements, copy number alterations) were determined and then reported for these patient samples. Actionable GA was defined as those identifying anti-cancer drugs on the market or in registered clinical trials (CT).

Results: There were 23 male and 2 female SSCC patients with a median age of 70 years (range 48-91 years). Fourteen (56%) SSCC were grade 2 and 11 (54%) grade 3. Fourteen (46%) SSCC were stage III and 11 (54%) were stage IV at time of sequencing.

A total of 161 alterations were identified for an average of 6.44 alterations per tumor with 25/25 (100%) of patients harboring at least one alteration. The dominant mutational signature reflects DNA damage due to ultraviolet light exposure, a known risk factor for SSCC. The most common non-actionable GA were alterations in *TP53* (88%), *NOTCH2* (20%) and *MLL2* (20%). Twenty-one (84%) of SSCC had at least 1 actionable GA with an average of 2.08 actionable GA per patient including mutation, amplification or homozygous deletion of *CDKN2A* (60%), *NOTCH1* (40%), *ERBB2* (12%), *FGFR3* (12%), *NF1* (8%), *NF2* (8%), *PIK3CA* (8%), *SMARCACB1* (8%), *BRAF* (4%), *BRCA1* (4%), *BRCA2* (4%), *CCND1* (4%), *EGFR* (4%), *HRAS* (4%), *PIK3R1* (4%), *PIK3R2* (4%) and *PTCH1* (4%).

Conclusions: More than three-quarters of SSCC patients harbored actionable GA which has the potential to treat high stage tumors with targeted therapeutic agents in addition or in replacement of the currently available treatment options.

520 Evaluation of Chromosomal Aberrations in Melanocytic Proliferations by *Fluorescence In Situ Hybridization*: A Study from Southern Part of Turkey

D Anlar, M Ergin, A Acikalin, E Kilic Bagir, O Diniguzel, A Yucel, S Zorludemir, I Tuncer. Cukurova University Medical Faculty, Adana, Turkey.

Background: Melanocytic proliferations consist of a heterogeneous group of lesions with different biologic and clinical behavior. It is usually easy to define the definite benign and malignant melanocytic lesions histopathologically. However, there is a gray zone group of lesions, of which histopathologic findings do not fit to either benign or malignant criteria. In the last years, molecular studies have become more of an issue to define melanocytic proliferations. In this study, we investigated the discriminator role of chromosome 6 and 11 aberrations by using fluorescence in situ hybridization (FISH).

Design: Study included 17 benign nevi, 29 malignant melanoma, and 28 gray-zone group, consisting of: 19 dysplastic nevi (17 dysplastic compound, 2 dysplastic junctional nevi), 5 spitz nevi, 3 atypical spitz nevi, and 1 cellular blue nevi. Four-color probe set targeting 6p25, 6q23, 11q13 and CEP6 was used for FISH.

Results: In 46 definite nevi and melanoma cases, sensitivity and specifity of FISH was 97.7% and 100%, respectively. Benign nevi group did not show any chromosomal aberrations. The most frequent chromosomal aberration was RREB (43%), and the following was CCND1 (28.4%). Six out of 28 (21.4%) gray-zone group cases were FISH (+). Three out of six FISH (+) cases showed recurrence. FISH (-) cases did not exhibit recurrence.

Conclusions: FISH has a discriminator role in diagnosis of benign nevi and melanoma. FISH positivity is a valuable parameter for the diagnosis of problematic area of the melanocytic proliferations. However, larger series of studies are needed to focus on contribution of FISH analysis to diagnosis of ambiguous melanocytic proliferations.

521 Caseating Granulomas in Cutaneous Leishmaniasis: TB or Not TB

J Aoun, R Habib, S Taraif, A Loya, R Mahfouz, I Khalifeh. American University of Beirut Medical Center, Beirut, NA, Lebanon; Shaukat Khanum Memorial Cancer Hospital and Research Centre, Lahore, NA, Pakistan; Saad Specialist Hospital, Al Khobar, NA, Saudi Arabia.

Background: Caseating granulomas are often associated with a mycobacterial infection (TB) and are thought to be exceedingly rare in cutaneous leishmaniasis (CL). However, no large series has accurately documented the incidence of caseating granulomas in CL.

Design: A multiregional cohort consisting of 317 patients with CL [Syria (157), Pakistan (66), Lebanon (47), Saudi Arabia (43), Ethiopia (2) and Iran (2)] was reviewed. Clinical [age, sex, disease duration, lesion type and geographic and anatomic location] and microscopic [presence of and type of granuloma, Ridley's parasitic index (PI) and pattern (RP)] data were documented. Presence of microorganisms was evaluated using special stains (GMS, PAS, AFB and Gram) and polymerase chain reaction (PCR) for TB and CL. All cases included in this study were confirmed as CL by PCR followed by restriction fragment length polymorphism analysis for molecular speciation and were negative for other organisms by all other studies performed. Categorical and continuous factors were compared for granuloma types using Chi-square, t-test or Mann-Whitney test as appropriate.

Results: Granulomas were identified in 195 (61.5%) cases of CL and these were divided to 49 caseating (25.2%), 9 suppurative (4.6%) and 137 tuberculoid without necrosis (70.2%). Caseating and tuberculoid granuloma groups were significantly different in terms of: the geographical source, with more cases harboring caseating granulomas in Saudi Arabia ($p<0.05$), and the distribution of their RP ($p<0.0001$) with a doubling RP3 in caseating (31% vs. 15%) as opposed to doubling of RP5 in tuberculoid (38% vs. 19%) granuloma group. Time needed to achieve healing (RP5) was notably shorter in tuberculoid vs. caseating group (4.0 vs. 6.2 months). Parasitic Index, CL species and other considered variables did not differ for the granuloma type groups.

Conclusions: In our multiregional cohort, a notable 15.5 % of all CL cases harbored caseating granulomas therefore; CL should be considered part of the differential diagnosis for cases with caseating granulomas in endemic regions, especially considering that the regions included in our cohort are also endemic for TB. Of note, cases of CL with caseating granulomas also showed a slower healing process, with no association with specific species, which may be due to worse host immune response in such cases.

522 Atypical Umbilical Nevi: Histopathologic Analysis with Comparison to Umbilical Melanoma

DP Arps, DR Fullen, MP Chan. University of Michigan, Ann Arbor, MI.

Background: Melanocytic nevi on the umbilicus have been described as a form of flexural nevi, with the most common feature reported as a "nested and dyshesive pattern". We have encountered a distinct type of umbilical nevi with more significant atypia and prominent lamellar fibrosis, which have not been previously reported. This study aimed to better characterize these nevi, and to identify the most helpful features in distinguishing them from umbilical melanoma.

Design: Seventy-nine umbilical nevi and 5 umbilical melanomas were identified in our archive. The nevi were screened for the presence of atypia and/or lamellar fibrosis. Histologic features were assessed in these atypical nevi and umbilical melanomas. Chi-square tests and t-tests were used to compare the results of the two groups.

Results: Nineteen umbilical nevi showing atypia and lamellar fibrosis were identified and designated as atypical umbilical nevi (AUN). The patients with AUN are mostly females (F:M=2.2:1) and range from 10 to 46 years old (average 29.7 years). Histologic features observed in AUN in descending order of frequency include: lamellar fibrosis (100%), bridging (100%), shoulder (94%), lentiginous growth (84%), significant junctional cytologic atypia (84%), poor circumscription (69%), adnexal involvement (42%), lymphocytic infiltrate (37%), and large round junctional nests with dyshesive pattern (21%). Lamellar fibrosis in AUN is similar to that described in dysplastic nevi but extends more broadly and deeply into the dermis. Some cases (42%) demonstrate minimal maturation within the areas of fibrosis, although complete maturation is invariably seen outside of the fibrotic zones. Compared to AUN, umbilical melanoma tends to affect older patients (average 45.8 years, $p=0.0088$) and is more likely to show confluence (80% vs. 0%, $p<0.0001$), prominent pagetoid spread (80% vs. 0%, $p<0.0001$), significant dermal cytologic atypia (100% vs. 5%, $p<0.0001$), dermal mitoses (60% vs. 0%, $p=0.0005$), and suprapapillary plate involvement (100% vs. 21%, $p=0.0113$). Follow-up data available for 14 AUN show no recurrence or metastasis in an average follow-up period of 48.9 months.

Conclusions: In addition to classic flexural nevi, a subset of umbilical nevi demonstrate characteristic lamellar fibrosis associated with architectural disorder and cytologic atypia. Recognition of the site-related atypical features would avoid overdiagnosis of melanoma. On the other hand, the findings of confluent growth, prominent pagetoid spread, severe dermal cytologic atypia, and dermal mitoses should elicit a high suspicion for umbilical melanoma.

523 Lymphovascular Invasion and Angiogenesis in Primary Cutaneous Melanoma – Correlation with Established Histopathologic Prognosticators and *BRAF* Status

PP Aung, JK Feller, S Yang, J Massaro, M Hernandez, R Yaar, R Singh, T Helm, M Mahalingam. BU, Boston, MA; BU School of Medicine, Boston, MA; UT Southwestern, Dallas, TX; Aurora Diagnostics, Greensboro, NC; Mount Sinai School of Medicine, New York, NY; NY State University, Buffalo, NY.

Background: While angiogenesis and lymphovascular invasion (LVI) are both associated with poor prognosis in primary cutaneous melanoma (PCM), the relationship between these two is unclear which, was the primary aim of our study. Additional aims included ascertaining a correlation of these with established histopathologic prognosticators and to the *BRAF* status.

Design: A total of 102 primary melanomas were assessed for; LVI (using double immunostaining with D2-40/S100 and H&E); MVD using the vascular endothelial growth factor (VEGFR)-2 (a VEGF autocrine growth factor receptor) and Endocan (a proteoglycan that binds select pro-angiogenic molecules) and, S100A13 (functionally essential for release of select proangiogenic molecules). Genotyping for *BRAF* was performed on all samples.

Results: Findings of statistical significance noted were the following; LVI, (by both methods) was significantly associated with ulceration and *BRAF WT* status ($p<0.05$ and 0.04 respectively), using VEGFR-2, an association was noted between mean intratumoral MVD with ulceration and tumoral VEGFR-2 expression ($p<0.05$ for both) as well as peritumoral MVD with the *BRAF* mutation ($p=0.05$), while only a trend towards statistical significance was noted between mean intratumoral and peritumoral MVD with tumor thickness ($p<0.08$ for both) and, using Endocan, a significant correlation was noted only between mean intratumoral MVD and tumoral Endocan expression ($p<0.0007$). A *BRAFV600E* mutation was noted in 21 (21%) and a non-*BRAFV600E* mutation noted in 10 (10%) cases. Double immunostaining increased detection rate of LVI by almost tenfold (46.5% *vs*. 4.9%, $p<0.0001$) with the sensitivity of H&E *versus* double staining for detecting LVI was 53% and 98% respectively.

Conclusions: Our findings indicate that antiangiogenic therapy may potentially be beneficial in select melanomas and may be used in conjunction with BRAF targeted therapy. We also underscore the utility of routinely using double immunostaining with D2-40 and S100 to detect LVI in melanoma. Last, but not least, the sizable prevalence of non-BRAFV600E mutations in our cohort indicates that BRAF therapy needs to target non-BRAFV600E mutations.

524 A Comprehensive Analysis of the Effects of MAPK Pathway Inhibition on the Immunological Properties of Melanoma Cells

A Boni, JL Fisher, TH Hampton, S Yan, LJ Tafe, GJ Tsongalis, MJ Turk, DW Mullins, CE Brinckerhoff, CE Fadul, MS Ernstoff. Dartmouth Hitchcock Medical Center, Lebanon, NH; Geisel Medical School at Dartmouth, Lebanon, NH.

Background: Metastatic malignant melanoma remains an incurable disease, but recent advances in innovative therapeutic modalities such as molecule tyrosine kinase inhibitors offer exciting new opportunities. In the treatment of melanoma, MAPK pathway inhibition has undergone extensive pre-clinical and clinical investigation. After several years of experience with these new drugs it has become clear that additional combinatory approaches are needed to achieve and sustain long lasting clinical remissions. Immunotherapy based approaches are strong candidates to partner with small molecules inhibitors and in the present study we sought to elucidate how MAPK inhibition influences the immunological characteristics of melanoma cells.

Design: We tested 33 human melanoma cell lines with a somatic mutation PCR array assay interrogating common point mutations in the AKT1, BRAF, cKIT, KRAS, HRAS, NRAS, MEK1, PIK3CA and PTEN genes. Cell lines were treated with either

vemurafenib (PLX4032, a BRAFV600E inhibitor) or U0126 (a MEK1/2 inhibitor) for 24 hours. Concentrations of the drugs were selected to achieve growth arrest without causing over apoptotic death. The cells were analyzed for production of 42 different cytokines and the expression of 9 surface immunomodulatory molecules and 5 antigens.
Results: The mutation status, as revealed by our PCR assay, was not predictive of any tested feature. MAPK pathway inhibition induced significant changes in the cytokine production levels in many of the tested cytokines. Responses to drug treatments were significantly associated with the BRAF mutation status of the cell lines. BRAF mutant lines responded differently from wild type lines to BRAF inhibition while MEK inhibition was less affected by BRAF status. Specifically, cytokines (including EGF, TGFα, G-CSF, Fractalkaline, IL7, IL8, PDGF-AA, GRO, IFN-2α and VEGF) showed a statistically significant change after pharmacologic treatment. No definite changes were observed in surface molecule expression including HLA- class I and II, PDL-1 and FAS. Antigen expression level changes, as detected by immunohistochemistry, were modest and only observed in a subset of cell lines for melanoma associated antigens *tyrp1* and *gp100*.
Conclusions: BRAF or MEK inhibition leads to changes in immunological properties of melanoma cells that are dependent on their BRAF mutation status. A clear understating of these effects will be helpful in designing highly effective combinatory treatments.

525 Diagnostic Value of T-Cell Receptor Gene Rearrangement Testing in Paraffin Embedded Skin Biopsies

AF Brown, MC Kinney, K Vadlamudi, Y Wang, H Fan. University of Texas Health Science Center, San Antonio, TX.
Background: BIOMED-2 PCR assays are useful in detecting clonal T-cell populations. The evaluation of abnormal lymphoid infiltrates in the skin is difficult and often requires clonality assays to confirm a diagnostic impression. We have compared the diagnostic value of TCRβ and TCRγ assays on skin biopsies from patients with suspected T-cell lymphoma (TCL).
Design: 354 formalin-fixed paraffin embedded (FFPE) skin biopsies from 2008-2012 were analyzed for TCR gene rearrangement using TCRβ (Vβ, Jβ, Dβ) and TCRγ (Vγ, Jγ) mutiplex PCR assays utlizing Gene Clonality Assay Kits (InVivoScribe Technology) followed by capillary gel electrophoresis (ABI 3120xl Genetic analyzer).
Results: Of 337/354 (95.2%) cases with successful extraction, 303/337 (89.9%) had non-equivocal results with 110 cases showing clonality (Table 1). 78/80 (98%) cases diagnosed as mycosis fungoides (MF) or non-MF TCL were TCRγ+, and 73/76 (96%) were TCRβ+ with 2/80 (2.5%) TCRγ- and 3/76 (4%) TCRβ-. 23/110 (20.9%) cases were designated as abnormal clonal T-cell infiltrate due to TCR clonality and histological features with 18/23 (78.3%) concerning for lymphoma. 7/110 (6.3%) cases with benign features were TCRγ+ with 5/7 also TCRβ+. Follow-up in 3/7 benign cases showed an abnormal immune state or hydroa vacciniforme, the remaining 4/7 cases resolved with no treatment or topical steroids. Repeat biopsy (≥2 sites) was performed in 37/110 (33.6%) of cases (29 MF, 1 TCL, 7 ACTCI) with confirmatory results in 88.2% TCRβ and 94.6% TCRγ. Equivocal results in 34/337 (10%) cases were due to inadequate peak height, oligoclonality, or non-duplication; 7/34 were found to express clonality on repeat biopsy.

Table 1-TCR rearrangement distribution in clonal cutaneous T-cell proliferations (n=110)

	γ+β+(%)	γ+β-(%)	γ-β+(%)	γ+βNT(%)
MF (n=62)	54 (87.1)	3 (4.8)	2 (3.2)	3 (4.8)
TCL, non-MF (n=18)	17 (94.4)	0 (0)	0 (0)	1 (5.6)
ACTCI (n=23)	13 (56.5)	3 (13)	5 (21.7)	2 (8.7)
Benign process (n=7)	5 (71.4)	2 (28.6)	0 (0)	0 (0)

Abbreviations: ACTCI, atypical clonal T-cell infiltrate; NT, not tested

Conclusions: DNA extraction and TCR analysis on FFPE small skin biopsies are successful in 95% of cases. Overall <5% of MF or other TCL appear to be falsely negative using both TCRγ and TCRβ assay. In a small percentage of cases, clonal populations were detected in "reactive" conditions particularly in patients with abnormal immune background (autoimmune disease, immunomodulating drugs). In about 20% of clonal cases where the diagnosis remains atypical, close clinical follow-up with repeat biopsies are indicated to further define the process.

526 Localized Lymphedema of Genital Areas: A Clinicopathologic Report of 18 Cases and Review of the Literature

L Carrillo, M Sangueza, S Suster, D Kazakov, D Kacerovska, M Michal, L Requena, JA Plaza. Medical College of Wisconsin, Milwaukee, WI; Sikls's Department of Pathology Charles University Medical Faculty Hospital, Pilsen, Czech Republic; Hospital Obrero, La Paz, Bolivia; Universidad Autonoma de Madrid, Fundación Jiménez Díaz, Madrid, Spain.
Background: Massive localized lymphedema is a rare reactive soft tissue pseudotumor that is strongly associated with morbid obesity. The relationship between simple lymphedema and massive localized lymphedema has not been well established pathologically, but most likely represents different end of spectrum of group of conditions related to chronic lymphatic obstruction. It presents as a giant swelling, with characteristic skin changes. Herein, we report 18 cases of this rare entity, highlighting their salient clinical and histopathologic features.
Design: Eighteen cases localized genital lymphedema form the basis of our study. The cases included 13 women and 5 men, aged from 25 to 68 years (mean: 46.5 years). Clinical data and follow-up information was obtained from the hospital records.
Results: Of the 18 cases, 12 patients were obese at diagnosis and one patiet had hidradenitis suppurative. Twelve patients presented with tumors involving the vulva (one of these patients presented with lower abdomen involvement), four patients with tumors in the penis (two of these patients presented with scrotal involvement), one patient with pure scrotal lesions, and one patient with pubis and perineal lesions. Grossly, the patients typically presented with numerous, multinodular papillomatous, confluent and discrete, skin-colored polyps that had been present for durations that ranged from 2 months to 6 years. Microscopically, all cases showed hyperplasia and papillomatosis of the epidermis. All lesions had a thickened papillary dermis with variable expansion by fibrosis and edema. The stroma was populated by reactive and multinucleated stromal fibroblasts. There was a patchy lymphoplasmacytic inflammatory response predominantly in a perivascular distribution. Lymphangectasia and microvascular proliferation were seen in all cases. Some cases also showed apocrine and eccrine ductal hyperplasia and squamous metaplasia of luminal cells forming micropapillae protruding into the lumens leading to partial obliteration.
Conclusions: Genital localized lymphedema is a rare pseudoneoplastic condition that has been relatively recently described and it has only rarely been mentioned in the literature. It should be considered in the etiology of skin tumors when asseing a polyp or mass with dermal edema, fibrosis and dilated lymphatics.

527 Ulceration, Breslow Thickness and the AKT-PI3K Pathway in Melanoma: Immunohistochemistry, Gene Expression Analysis, and Next Generation Sequencing

E Castro-Echeverry, RT Zreik, A Werlang-Perurena, K Walker, L Watson, D Jupiter, MP Fernandez, A Rao. Scott & White Hospital, Temple, TX.
Background: Ulceration and Breslow thickness are morphologic predictors of prognosis in melanoma. While there are published studies evaluating molecular mapping in melanomas, little work has been done on mapping molecular changes in morphological subsets of melanomas. In the present study, we matched ulcerated and non-ulcerated melanomas by Breslow thickness and performed next generation sequencing, EMT and PI3K signaling pathway gene expression array analysis and immunohistochemistry to determine the relationship between the PI3K-AKT pathways with ulceration and thickness.
Design: Ulcerated and non-ulcerated melanomas were matched for Breslow thickness. Tumor was macrodissected from FFPE and cDNA was extracted from macrodissected tissue and analyzed with the RT^{2}Profiler PCR ARRAY (Rotor-Gene) Human EMT kit and the PI3K-AKT signaling expression arrays consisting of 80 genes each (Qiagen). Next generation sequencing was performed using the Ion AmpliSeq™ Comprehensive Cancer Panel (400 genes) on the Ion Torrent PGM (Life Technologies) to identify possible SNP variants involved in the pathway. Protein expression of AKT-1 and p-AKT-1 in ulcerated and non-ulcerated melanomas was evaluated by immunohistochemistry (Santa Cruz Biotechnologies).
Results: A total of 17 matched pairs of ulcerated and non-ulcerated melanomas were analyzed in this study, with thickness ranging from 0.67 mm to 14 mm. Gene expression arrays showed a linear relationship between AKT-1 expression and thickness in ulcerated melanomas(R^2 of 0.97). Heat-map analysis of the PI3K-AKT pathway suggests an association between overexpression and decreased survival. By immunohistochemistry, there was correlation (R^2 of 0.975) between nuclear staining for p-AKT-1 and thickness in ulcerated melanomas with variation in leading edge staining and distribution. Analysis of next generation sequencing data identified single nucleotide pleomorphisms unique to ulcerated melanomas in 14 different genes, with variable relationships to the PI3K-AKT pathway. These subsets were wildtype for BRAF, but showed mutations and increased expression of HRAS and MITF.
Conclusions: A linear relationship between AKT-1 gene expression and thickness was identified in ulcerated melanomas which were predominantly negative for BRAF mutations and showed HRAS overexpression. Protein expression and phosphorylation by immunohistochemistry was complex with differential staining noted between ulcer margins and leading edge distribution.

528 Cutaneous PEComas: An Expansion of the Morphologic Spectrum and a Subset with TFE3 Positivity

AL Cheah, F Maclean, S O'Toole, T Selinger, SD Billings. Cleveland Clinic, Cleveland, OH; Douglass Hanly Moir Pathology, Sydney, NSW, Australia; Royal Prince Alfred Hospital, Sydney, NSW, Australia.
Background: PEComas are unusual mesenchymal tumors that show both melanocytic and smooth muscle differentiation. PEComas arising as primary cutaneous neoplasms are rare. To better understand these tumors, we examined the clinical and pathologic features of 10 primary cutaneous PEComas from a single institution.
Design: Primary cutaneous PEComas were retrieved from the consultation files at Cleveland Clinic between 2003 and 2013. The clinical, morphologic and immunohistochemistry features of these cases were analyzed.
Results: Six patients were male and four were female. The age of presentation ranged from 10 to 84 years (median 47y). Sites of involvement included lower extremity (6), upper extremity (2), back (1) and scalp (1). Size ranged from 0.9 cm to 6.5 cm. 8 were dermal, 4 of which pushed into the subcutis, and 2 were entirely subcutaneous. 1 case was encapsulated. All showed nested to fascicular growth pattern and were composed of epithelioid to spindled cells with pale eosinophilic to clear cytoplasm. 3 cases were classified as histologically malignant; these cases showed considerable pleomorphism, prominent nucleoli and mitotic counts of 1 to 21/10 HPF. Immunohistochemisty results are summarized below. In three patients for whom follow-up data was available (8 to 31 months), there were no recurrences.

Immunohistochemistry Profile of Cutaneous PEComas

Case	HMB45	Melan-A	MiTF	Tyrosinase	SMA	Desmin	TFE3
1	+	-	-	+	-	-	+ (weak)
2	+	+	NP	NP	+	+	-
3	-	+	+	NP	+	-	-
4	NP	+	-	NP	+	NP	NP
5	+	-	+	-	-	+	-
6	+	NP	NP	NP	-	NP	-
7	+	+	+	+	-	-	+ (strong)
8	+	+	+	+	+	+	-
9	+	-	NP	-	-	NP	NP
10	+	-	NP	NP	NP	-	-

NP: Not Performed. All cases negative for S100 protein except case 5.

Conclusions: Our results expand the morphologic spectrum of cutaneous PEComas to include malignant histology. Similar to their counterparts in deep locations, a subset of cutaneous PEComas is positive for TFE3.

529 The Evaluation of Primary and Metastatic Cutaneous Lesions with Clear Cell Histology Using Squalene Synthase (SQS), Progesterone Receptor Membrane Component-1 (PGRMC-1) and Perilipin (PLIN1)

P-L Chen, W-S Chen, D Lu, LP Dehner, D Ivan. MD Anderson Cancer Center, Houston, TX; Washington University in St. Louis, St. Louis, MO; St. Luke's Hospital, Chesterfield, MO.

Background: Sebaceous carcinoma (SebCA) is an aggressive and potentially fatal adnexal neoplasm arising from the sebaceous glands. The diagnosis of SebCA can be challenging due to its rarity and its histologic mimicry to a variety of benign and malignant cutaneous lesions. When evaluating cutaneous tumors with clear/vacuolated cytoplasm, a variety of primary and metastatic cutaneous lesions enter into the diagnostic consideration, including sebaceoma, SebCA, basal cell carcinoma (BCC) and squamous cell carcinoma (SCC) with clear cell feature, and metastatic clear cell renal cell carcinoma (met ccRCC). Unfortunately, markers unique for sebaceous differentiation are limited. As sebaceous glands are steroidogenic and lipogenic organs that produce sebum, we sought to test the utility of three lipid synthesis/processing proteins, namely PGRMC-1, SQS and PLIN1, in differentiating SebCA from other primary and metastatic cutaneous tumors with clear cell histology.

Design: Anti-SQS, PGRMC-1 and PLIN1 antibodies were applied to 23 SebCA, 14 sebaceomas, 14 BCC with clear cell feature, 1 SCC with sebaceous differentiation and 11 met ccRCC to the skin using FFPE tissues.

Results: In SebCA, SQS, PGRMC-1 and PLIN1 showed strong cytoplasmic vesicular staining in tumor cells (96%, 96% and 91%, respectively). When used in combination, SQS, PGRMC-1 and PLIN1, showed a sensitivity of 100% for SebCA. In sebaceomas and SCC with sebaceous differentiation, SQS, PGRMC-1 and PLIN1 strongly highlighted tightly clustered lipid vesicles in the sebocytes. In BCC with clear cell feature, SQS, PGRMC-1 and PLIN1 are negative in the tumor cells. Even though faint cytoplasmic blush was observed with PGRMC-1 and SQS in some cases, the discrete cytoplasmic vesicular staining pattern specific for sebaceous differentiation was not seen. Lastly, SQS and PLIN1 were negative in met ccRCC (100% and 91%, respectively), while PGRMC-1 showed faintly membranous and cytoplasmic positivity in 70% of cases.

Conclusions: 1) SQS, PGRMC-1 and PLIN1 are excellent markers for sebaceous differentiation. 2) SQS has a higher specificity than PGRMC-1 and PLIN1. 3) When used in combination, SQS, PGRMC-1 and PLIN1 can serve as a useful diagnostic adjunct in distinguishing sebaceous tumors from other cutaneous lesions with clear cell histology.

530 Actinic Cheilitis Versus Inflammatory Dermatoses of the Lip: Clinicopathologic Review of 93 Lip Biopsies with Significant Inflammation

E-YK Choi, MP Chan. University of Michigan, Ann Arbor, MI.

Background: Recognition of actinic cheilitis (AC) is important as it carries a high risk of progressing to squamous cell carcinoma. One of the characteristics of AC is a variably intense inflammatory infiltrate, a feature shared by various inflammatory dermatoses of the lip such as lichen planus (LP). Reactive atypia seen in inflammatory conditions further adds to the diagnostic challenge. This study aimed to find the most useful clinical and histologic features in distinguishing AC from inflammatory dermatoses.

Design: Lip biopsies taken from 2003-2013 were retrieved from our archive. A total of 93 cases demonstrating significant band-like inflammation were selected. Clinical data were obtained from electronic medical records. Histologic features were assessed in all cases. Data were compared between groups using Chi-square tests and t-tests.

Results: Seventy-two cases demonstrate significant squamous dysplasia consistent with AC, of which 8 cases (11%) also reveal classic features of LP in the background skin/mucosa. The remaining 21 cases of inflammatory dermatoses include classic or erosive LP (62%), lichenoid hypersensitivity reaction (19%), Zoon's cheilitis (9%), hypertrophic lupus erythematosus (5%), and Behcet's syndrome (5%). Features that are significantly associated with AC include squamous atypia ($p=0.0007$), atypical parakeratosis ($p=0.0038$), and prior history of non-melanoma skin cancer (NMSC) ($p=0.0136$). On the other hand, colloid bodies ($p=0.0025$) and basal squamatization ($p=0.0096$) are significantly associated with inflammatory dermatoses. The infiltrates in ulcerated or eroded samples show a higher percentage of plasma cells compared to non-ulcerated samples (44% vs. 14%; $p<0.0001$). Solar elastosis, acantholysis, hypergranulosis, ulcer, spongiosis, and individual apoptotic keratinocytes do not help in the differential diagnosis of AC versus inflammatory dermatoses ($p=0.1614$-0.8752). Clinically, no significant difference is observed in the patients' age, sex, site of lesion (lower vs. upper lip), and smoking history between the two groups.

Conclusions: Distinction between AC and inflammatory dermatoses remains a diagnostic challenge. Atypical parakeratosis is the most useful clue to AC besides squamous atypia. A clinical history of prior NMSC also favors AC. LP comprises the majority of inflammatory dermatoses of the lip, and its diagnosis is best supported by the presence of colloid bodies and basal squamatization.

531 Eccrine Angiomatous Hamartoma: A Clinicopathologic Review of 14 Cases

LN Clark, P Nagarajan, SB Peters, S Bayliss, LP Dehner, M Gupta, AA Gru. The Ohio State University Wexner Medical Center, Columbus, OH; Washington University, Saint Louis, MO.

Background: Eccrine angiomatous hamartoma (EAH) is a rare, benign nodular lesion typically presenting at birth or in young children. Histologically, there is an increase in vascular and eccrine elements with an abnormal distribution. EAH has been characterized as a hamartoma; however, an alternative view, suggests that it is a vascular lesion and the abnormalities of eccrine units are a secondary phenomenon. Support for this theory includes hyperplasia of other dermal constituents such as nerve, fat, and hair follicles. Clinically, the differential diagnoses include vascular lesions or dysplastic nevi.

Design: Archive files at two university hospitals (Barnes-Jewish Hospital and Wexner Medical Center) were searched for ten years for cases of EAH. Fourteen cases were identified and reviewed for accuracy of diagnosis.

Results: The age of the patients ranged from 3 days to 84 years old. A male predominance was observed with a ratio of 9:5, with the majority occurring on the extremities (10 extremities: 2 scalp: 3 trunk/buttock). Clinical diagnoses included vascular malformations, atypical nevus, and SCC (data for 4 were not available on review). Two of the children had two lesions. The lesions were slightly more common on the upper extremity than the lower extremity at a ratio of 4:3. Microscopic evaluation showed an increase in eccrine (100%) and vascular elements (57%). Thirty-five percent (35%) of the cases show vascular dilatation in the papillary dermis. There was an abnormal distribution of the eccrine ducts in all the cases. The predominate associated inflammatory infiltrate, seen in 5/14 cases, consisted of minimal to mild perivascular lymphocytes and histiocytes. Three of the lesions had plasma cells and one case had concurrent eosinophils. A single case showed prominent acanthosis in addition to the vascular dilatation. The oldest individual had an overlying well-differentiated squamous cell carcinoma.

Conclusions: EAH is a rarely encountered hamartomatous lesion most commonly found in children; however, in our review five patients were adult-age. Interestingly, we found the association of a squamous cell carcinoma with EAH which, to our knowledge, has not been previously documented. Three cases showed extensive vascular dilatation in the superficial dermis, which raise some consideration for a vascular anomaly in the pathogenesis of the lesion.

532 Immunohistochemistry for Merkel Cell Polyomavirus Large T Antigen (CM2B4) and Neurofilament Protein Adds Little Value to CK20 and TTF-1 in the Distinction of Cutaneous and Visceral High-Grade Neuroendocrine Carcinomas

TW Czeczok, JL Hornick, AM Bellizzi. University of Iowa, Iowa City, IA; Brigham and Women's Hospital, Boston, MA.

Background: The differential diagnosis of a high-grade neuroendocrine carcinoma (HGNEC) in the skin includes Merkel cell carcinoma (MCC) and spread from a visceral small cell carcinoma, of either lung (SCLC) or extrapulmonary (ESCC) origin. Immunohistochemistry (IHC) for CK20 (+ in MCC/- in SCLC) and TTF-1 (+ in SCLC/- in MCC) is well-vetted in this setting, though data for ESCC are limited. IHC for the large T antigen of Merkel cell polyomavirus (CM2B4) and neurofilament protein (NF) have been advanced as additional MCC markers. Whether CM2B4 adds value beyond CK20 is not established, and, while most studies have shown good sensitivity/specificity of NF for MCC, rates of NF-positivity in SCLC have ranged from 0-69%, and expression in ESCC is not well-established.

Design: Tissue microarrays were constructed from 39 MCCs, 24 SCLCs, and 18 ESCCs. IHC for CK20, TTF-1, CM2B4, and NF was performed. For NF, staining was performed with the SMI-31 and 2F11 clones; for the latter, staining was repeated in 2 laboratories. Any nuclear (TTF-1, CM2B4) or cytoplasmic (CK20, NF) staining was considered positive. Sensitivity and specificity were calculated.

Results: Rates of positivity for the various markers by tumor type are summarized in the Table.

Protein Expression in HGNECs (%)

	MCC (n=39)	SCLC (n=24)	ESCC (n=18)
CK20	90	8	6
TTF-1	5	96	44
CM2B4	38	0	0
2F11 (lab 1)	67	17	6
2F11 (lab 2)	23	0	0
SMI-31	33	13	0

CK20 was 90% sensitive/93% specific for a diagnosis of MCC, while TTF-1 was 74% sensitive/95% specific for a diagnosis of visceral HGNEC (96% sensitive for SCLC). While CM2B4 was 100% specific for MCC, it was only 38% sensitive, with staining only seen in CK20(+)/TTF-1(-) cases. The sensitivity of NF expression for MCC varied significantly based on laboratory and clone (23-67%), as did specificity (88-100%). 2F11 (lab 1) and SMI-31 were each positive in 2 of 2 CK20(+)/TTF-1(+) and 1 of 4 CK20(-)/TTF-1(-) MCCs.

Conclusions: This study affirms the value of CK20 and TTF-1 IHC in distinguishing cutaneous and visceral HGNECs. Although specific for MCC, CM2B4 was only positive in cases that were also CK20(+)/TTF-1(-), limiting its utility. NF is occasionally expressed by visceral HGNECs, mainly of lung origin; results are highly influenced by clone and the laboratory performing the stain. Addition of NF IHC may be useful in occasional CK20(+)/TTF-1(+) and CK20(-)/TTF-1(-) tumors, where positive staining supports a diagnosis of MCC.

533 Shared Clonality in Distinctive Lesions of Lymphomatoid Papulosis and Mycosis Fungoides Occurring in the Same Patients Suggests a Common Origin

MM De la Garza Bravo, KP Patel, JL Curry, CA Torres-Cabala, DS Ivan, J Medeiros, VG Prieto, M Duvic, MT Tetzlaff. Houston Methodist Hospital, Houston, TX; The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Lymphomatoid papulosis (LyP) lies within the spectrum of cutaneous CD30-positive lymphoproliferative disorders. LyP is characterized clinically by clusters of small papules which undergo spontaneous regression, and these papules exhibit an array of histopathologic patterns. Approximately 10-15% of patients with LyP develop other lymphomas, including mycosis fungoides (MF), cutaneous anaplastic large cell lymphoma and Hodgkin lymphoma, suggesting a biological relationship among these clinically distinctive diseases.

Design: We reviewed the clinical, histopathologic, and immunophenotypic (CD3, CD4, CD8, and CD30) features of 11 patients (2009-2013) diagnosed with both LyP and MF. T-cell receptor (TCR) gene rearrangement studies were performed to assess the TCR-beta chain gene *(TCRB)* and TCR-gamma chain gene *(TCRG)* in all 11 patients.

Results: We studied 32 biopsies (14 LyP and 18 MF) from 11 patients. LyP preceded MF in 7 patients, followed MF in 3 and presented concomitantly with MF in 1 patient. LyP lesions were classified as type A (10), type B (1), type C (2) and type D (1). All cases of MF were characterized clinically by patch/plaque disease and were stage I or II at the time of diagnosis. Monoclonal TCR gene rearrangements were detected in 13 LyP lesions from 10 of 11 patients (including 13 *TCRG* and 9 *TCRB*) and in 14 MF lesions from 9 of 11 patients (including 13 *TCRG* and 11 *TCRB*). Nine of 11 patients exhibited identical clones in both their LyP and MF lesions; non-overlapping clones were not identified among any of these lesions. We failed to detect monoclonal TCR gene rearrangements in 1 LyP lesion from 1 patient and 4 MF lesions from 2 patients.

Conclusions: We demonstrate a clonal relationship between LyP and MF. This finding suggests a common origin between these two processes and that additional mechanisms determine whether an aberrant T-cell clone will progress into LyP versus MF. Laser capture microdissection studies are underway to delineate the cell type(s) responsible for shared clonality among lesions of LyP and MF.

534 GATA-3 Is Frequently Expressed in Benign and Malignant Epidermal and Cutaneous Adnexal Neoplasms

MN de Peralta-Venturina, BL Balzer, DP Frishberg, RB Mertens. Cedars-Sinai Medical Center, Los Angeles, CA.

Background: GATA-3 is a relatively specific marker for carcinomas of breast and urothelial origin. Recently, GATA-3 has been shown to also be expressed in various salivary gland tumors. Although GATA-3 is known to play a role in epithelial differentiation in the skin, the expression of GATA-3 in cutaneous epithelial neoplasms (CENs) has not been reported. The objective of this study was thus to examine the expression of GATA-3 in a wide variety of benign and malignant CENs.

Design: Immunohistochemical staining for GATA-3 was performed on 140 CENs from the pathology archives of Cedars-Sinai Medical Center. Extent of staining was graded as 0 (negative), 1+ (≤10%), 2+ (11-50%), or 3+ (>50%); intensity was graded as weak (1+) or strong (2+ or 3+).

Results: Benign tumors (n=99): GATA-3 was routinely expressed (usually 2+ or 3+, strong) in clear cell acanthoma, trichofolliculoma, trichoepithelioma, trichilemmoma, sebaceous adenoma, sebaceoma, apocrine hidrocystoma, apocrine tubular papillary adenoma, hidradenoma papilliferum, and syringocystadenoma papilliferum. Hidradenomas exhibited variable staining for GATA-3. Most cases of poroma, syringoma, chondroid syringoma, cylindroma, and spiradenoma were negative or only focally (1+) and weakly positive for GATA-3. Focal (1+ or 2+) weak to strong staining for GATA-3 was present in all pilomatrixomas. **Malignant tumors (n=41):** Basal cell carcinomas (BCC, n=14) and sebaceous carcinomas (SEBCA, n=6) were generally positive for GATA-3 (usually 3+, strong in BCC; variably 1+ to 3+ and usually weak in SEBCA). Two of eight squamous cell carcinomas were negative for GATA-3, though most exhibited positivity of variable extent (1+ to 3+) and intensity (weak to strong). The one apocrine carcinoma and both mucinous carcinomas exhibited 3+, strong staining for GATA-3, whereas the one eccrine carcinoma and the one adenoid cystic carcinoma were both negative. Two of three microcystic adnexal carcinomas were positive for GATA-3 (2+, weak or strong staining). One of five Merkel cell carcinomas exhibited 1+, weak staining for GATA-3; the remainder were negative.

Conclusions: GATA-3 is expressed in a wide variety of benign and malignant CENs. In addition to breast, urothelium, and salivary gland, the differential diagnosis of a metastatic tumor of unknown primary origin which expresses GATA-3 should also include a carcinoma of epidermal or cutaneous adnexal origin. In particular, in a woman with metastatic carcinoma of unknown primary origin in an axillary lymph node, immunoreactivity for GATA-3 should not be construed as proof of mammary origin.

535 GATA3 Immunohistochemistry Expression in Primary Cutaneous Tumors and Breast Carcinomas Metastatic to the Skin

G Deftereos, JM Uchin, JF Silverman, U Krishnamurti. Allegheny General Hospital, Pittsburgh, PA.

Background: GATA3 (GATA binding protein 3) is a transcription factor that plays a role in cell proliferation and differentiation. GATA3 expression is known to be a useful immunohistochemical (IHC) diagnostic marker for the identification of breast carcinoma (BC), especially Luminal A subtype. Previous studies have suggested that it plays an important role in stem cell lineage determination in skin, but scant data is available on the expression of GATA3 in primary cutaneous neoplasms. We investigated GATA3 expression in primary cutaneous tumors and compared its IHC expression to BC metastatic to the skin.

Design: 76 cases including 10 BC metastatic to skin, 17 basal cell carcinomas, 9 basosquamous carcinomas, 8 Merkel cell carcinomas, and 20 eccrine, 3 apocrine and 9 pilar adnexal neoplasms were immunostained for GATA3 (cat. #: HG3-31, sc-268, Santa Cruz Biotechnology; Leica Bond III stainer; Leica's Refine DAB kit detection). Stain intensity was graded as 1+, 2+ and 3+ and H-scores were calculated by multiplying the intensity with the percentage of tumor cells staining. GATA3 staining was compared between BC and the various skin neoplasms.

Results: GATA3 stained normal squamous epithelium and hair follicles, while normal eccrine adnexal structures were negative. All 10 cases of BC metastatic to skin were positive for GATA3, with strong diffuse staining in all 8 ER positive cases and variable less-intense staining in 2 ER negative cases. Variable positivity for GATA3 was present in all 26 cases of basal cell carcinoma (regardless of subtype) and basosquamous carcinomas. Apocrine and pilar tumors showed variable positivity in all cases, including 2 cases of desmoplastic trichoepithelioma. Only 4/20 eccrine tumors showed weak, focal staining, while all 8 Merkel cell carcinomas were negative for GATA3.

Conclusions: 1. Immunopositivity for GATA3 is seen in a variety of primary cutaneous tumors with variable intensities in basal cell carcinoma, basosquamous carcinomas, pilar and apocrine adnexal tumors, while eccrine adnexal tumors and Merkel cell carcinoma are negative. 2. Pathologists should be aware of the GATA3 immunoreactivity in adnexal neoplasms, especially in cases where metastatic BC is considered in the differential diagnosis. 3. Interpretation of GATA3 positivity can be especially challenging in partial and/or superficial biopsies of entities such as desmoplastic trichoepithelioma which can also morphologically resemble a metastasis from a well-differentiated breast carcinoma.

536 Desmoplastic Melanoma: A Clinicopathologic Review and Subclassification of 90 Cases

JC Fanburg-Smith, V Papavero, AK Esnakula, J Hallman. Sibley Memorial Hospital of Johns Hopkins Medicine, Washington, DC; Armed Forces Institute of Pathology, Washington, DC; Washington University, St Louis, MO.

Background: Desmoplastic melanoma (DM) is rare; there are no large series to study morphology. DM can mimic scar, benign fibrous histiocytoma, schwannoma and malignant peripheral nerve sheath tumor, with bland lesions difficult to recognize as malignant. Misdiagnosis rate is high. Only half DM have BRAF-mutations. We wanted to classify morphologic observations and aid in DM diagnosis.

Design: Cases coded as DM were reviewed, tabulated, and reclassified.

Results: 90 cases were included and divided into 2 groups, based on cellularity. Deceptive DM (DDM, n=43) were sparsely cellular with bland scar-like appearance and mild atypia. Obvious DM (ODM, n=47) had moderate to marked cellularity and atypia. Overall, there were 70 males (M) and 20 females (F), twice as many F in ODM than DDM, equal M. Patient ages ranged from 28-106 (mean 67.5) years. Tumor sizes ranged from 0.8-12cm, mean 3.8cm for DDM and 6.1cm for ODM. All DM were spindled, 85% showed solar elastosis. Lymphoid response (LR) was present in both groups. DDM was bland, myoid-appearing with single cells invading dermis at tumor edge, best observed on S100. Mitotic activity was always identified (at least 1 mitosis/mm2) but scarce, and there were adnexal-sparing, absent necrosis, and easily identifiable perineurial invasion (PNI). ODM invaded subcutis, deep fascia, and rarely skeletal muscle; 3 had predominant rhabdoid features. ODM did not have as pronounced PNI as DDM, due to increased cellularity, but showed deep neural hypertrophy, 50% with brisk mitotic activity including atypical forms, and occasional deep necrosis. Only 10 DDM and 8 ODM (20%) revealed overlying epidermal involvement (i.e., melanoma-in-situ). All cases were diffusely strongly S100 protein positive, yet only 3 cases had focal HMB45, tyrosinase, Melan-A, and mitf. Initial follow-up: 5 DM had documented recurrences; 1 DDM and 2 ODM metastasized.

Conclusions: DM can be divided into DDM and ODM. Features to assist in diagnosis of DDM include bland myoid-appearing cells notably infiltrating tissue edge, rare mitotic activity, solar elastosis, PNI, adnexal-sparing, LR, and diffuse, strong S100 positivity. DDM were smaller and less frequently F with more obvious PNI than ODM. DM generally do not demonstrate melanoma markers. Awareness of details of DDM and ODM, especially with 80% absent epidermal involvement, can separate DM from other neoplasms.

537 Increased Expression of Melanoma Stem Cell Marker CD271 in Metastatic Melanoma to the Brain

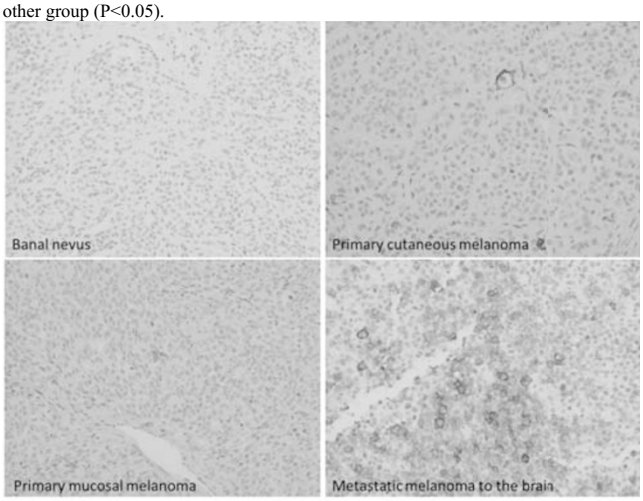
R Guo, J Chen, CZ Liu, K-M Fung, LA Hassell. University of Oklahoma Health Sciences Center, Oklahoma City, OK; University of Wisconsin School of Medicine and Public Health, Madison, WI.

Background: Human melanoma contains multipotent stem cells that express the neural crest stem cell marker CD271. CD271-expressing melanoma cells in murine xenografts give rise to metastatic tumor. However, a comprehensive clinical investigation of its role in different stages of melanomagenesis has not been well studied.

Design: We studied CD271 expression with immunohistochemistry in 11 cases of banal melanocytic nevus, 9 cases of primary cutaneous melanoma, 10 cases of primary mucosal melanoma, 5 cases of metastatic melanoma in regional lymph node, and 11 cases of metastatic melanoma in the brain. In addition, 9 cases of metastatic high-grade adenocarcinomas from breast and lung to the brain are also studied as controls. The staining is scored based on the number of positive cells and analyzed statistically by student *t*-test.

Results: The distribution of CD271 positive cells is most commonly as scattered single cells, less frequently as small clusters (<20 cells), occasionally as large clusters (>100 cells), rarely as very large cluster (>500 cells), or in mixed pattern. All banal melanocytic nevi show negative to focal equivocal staining. Primary melanomas show variable patterns, including 4 cases of negative to weakly positive staining, 3 cases of moderately positive staining and 2 cases of strongly positive staining. Mucosal melanomas are mostly negative except 3 cases of moderate positivity. Cases of lymph

node metastasis range from negative to moderate positivity. In contrast, 7 out of 11 cases of brain melanoma metastasis show strong positivity, and the rest of 4 cases are moderately positive. CD271 is negative to weakly positive in majority cases of brain metastasis from lung and breast carcinoma except one case. Statistically CD271 is significantly more highly expressed in brain melanoma metastasis compared to any other group (P<0.05).



Conclusions: The above findings suggest CD271 expression is specifically increased in metastatic melanoma to the brain. Further prospective study for the role of CD271 in prediction of melanoma brain metastasis as well as prognosis assessment will be of great clinical significance.

538 5-Hydroxymethylcytosine (5hmC) as an Ancillary Aid in the Diagnosis of Melanocytic Lesions

TT Ha, M Wang, S Hamarsheh, AA Andea. University of Michigan Health System, Ann Arbor, MI.

Background: 5-hydroxymethylcytosine (5hmC) is a marker of DNA methylation and has been recently reported to be lost in melanoma. However, little is known about the diagnostic utility of this marker in melanocytic lesions. The goal of the current study is to evaluate the expression of 5hmC and its diagnostic utility in benign and malignant melanocytic neoplasms, including spitzoid tumors.

Design: A total of 77 cases were analyzed including 29 melanomas (7 superficial spreading, 3 nodular, 4 lentigo maligna, 1 desmoplastic, 3 acral lentignous, 2 nevoid, 2 spitzoid, 1 pigment synthesizing, 2 unclassified, and 4 metastatic), 41 nevi (7 compound, 9 intradermal, 2 congenital, 3 Clark's, 2 blue nevi, 2 Spitz nevi, 14 atypical Spitz nevi, 1 atypical deep penetrating nevus, 1 atypical nevus with ancient change), and 7 atypical spitzoid tumors of undetermined malignant potential. Formalin-fixed, paraffin-embedded sections were immunolabeled with polyclonal anti-5hmC antibody (1:500). Nuclear staining intensity was scored as 0 (negative), weak (1), moderate (2), and strong (3) with respect to the epidermis as an internal control. Cases with staining intensity of 1, 2, and 3 in at least 20% of tumor volume were considered positive.

Results: While 88% of nevi showed positive 5hmC immunolabeling, only 31% of melanoma did (p<0.0001). Furthermore, in lesions that labeled with 5hmC, the average positive lesional cells were 27% in melanoma and 76% in nevi (p<0.0001). Of note, 5hmC was completely absent in nodular, lentigo maligna and desmoplastic melanoma but was positive in 47% of the remaining melanoma subtypes and metastases (33-57%). Within the nevus group, 100% of the conventional nevi labeled with 5hmC compared to 87% of the non-Spitz atypical nevi. Within the spitzoid group, 100% of Spitz nevi, 79% of atypical Spitz nevi, and 57% of atypical spitzoid tumors were positive.

Conclusions: In conclusion, immunoexpression of 5hmC can aid in the differential diagnosis of benign and malignant melanocytic neoplasms. In the context of spitzoid lesions, loss of 5hmC labeling may lend additional support towards the diagnosis of atypical Spitz tumor or spitzoid melanoma. Furthermore, the incremental decrease in the extent of 5hmC labeling is inversely related to the degree of atypia and implicates a role for 5hmC in the tumorigenesis and progression of melanocytic neoplasms.

539 Expression of the p40 Isoform of p63 Has High Specificity for Cutaneous Sarcomatoid Squamous Cell Carcinoma Relative to Histologic Mimics

TT Ha, S Chen, D Arp, RM Patel, SL Carskadon, J Siddiqui, DR Fullen, N Palanisamy, PW Harms. University of Michigan Health System, Ann Arbor, MI.

Background: p40 (ΔNp63), an isoform of p63, has oncogenic properties through its inhibitory function of p53. In cutaneous spindle cell neoplasms, p63 is one of the most reliable markers for sarcomatoid squamous cell carcinoma (S-SCC). While p40 has superior specificity compared to p63 for pulmonary squamous cell carcinoma, its sensitivity and specificity in cutaneous S-SCC has not been investigated. The goal of the present study is to evaluate the expression of p40 in S-SCC as well as its utility in the differential diagnosis of cutaneous spindle cell neoplasms, in particular atypical fibroxanthoma (AFX) and desmoplastic melanoma (DM).

Design: Tissue sections of immunohistochemically-characterized AFX (14), S-SCC (10), and DM (7) as well as conventional SCC (8) were immunolabeled with p40 (1:8000). Staining conditions of p40 were validated on pulmonary SCC (2), pulmonary adenocarcinoma (2), and normal skin. Tumors were scored for nuclear staining intensity (negative, weak, or moderate), distribution, and approximate percentage of positive tumor cells.

Results: More than half of S-SCC (6/10; 60%) exhibited moderate nuclear p40 positivity in 20-90% of tumor cells. An additional case showed focal weak positivity, and the 3 remaining cases were negative. p63 and cytokeratin (CK) expression were reviewed in a subset of cases and found to be positive in 5/6 (83%) and 6/8 (75%) cases of S-SCC, respectively. Of the p63-positive S-SCC, 2 were negative for p40 expression. On the other hand, only 1 of the CK-positive cases failed to stain for p40. All conventional SCC expressed p40 (8/8; 100%). p40 was completely absent in immunohistochemically-confirmed DM (0/7 cases), and demonstrated only rare weak nuclear staining in one case of AFX (1/14).

p40 Expression in Cutaneous Spindle Cell Neoplasm and Conventional Squamous Cell Carcinoma

DIAGNOSIS	p40 Positive/Total Cases (%)	p40 Intensity
SCC, conventional	8/8 (100)	Moderate
SCC, sarcomatoid	7/10 (70)	Moderate (6/10); Focal, weak (1/10)
Atypical fibroxanthoma	1/14 (7)	Focal, weak
Desmoplastic melanoma	0/7 (0)	Not applicable

SCC - squamous cell carcinoma

Conclusions: In conclusion, p40 is relatively specific for distinguishing sarcomatoid squamous cell carcinoma from atypical fibroxanthoma and desmoplastic melanoma, but may be less sensitive than p63. Further studies are needed to identify whether p40 may be expressed in other cutaneous spindle cell neoplasms.

540 p40 Improves Diagnostic Specificity between Atypical Fibroxanthoma and Poorly Differentiated Squamous Cell Carcinoma

SA Henderson, JL Curry, CA Torres Cabala, DS Ivan, RL Bassett, VG Prieto, MT Tetzlaff. The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: The diagnosis of atypical fibroxanthoma (AFX) remains controversial since it relies on the exclusion of other morphologically similar malignant spindle cell tumors. In particular, spindle cell squamous cell carcinoma (SpSCC) is distinguished from AFX based mostly on the absence of keratin immunoreactivity in AFX; however, SpSCC occasionally lacks detectable keratin expression, underscoring the need for other distinctive markers. p63 expression was initially specific for SpSCC; however, anti-p63 also highlights a subset of AFXs. Recent studies in lung carcinomas have demonstrated anti-p40 (recognizes a splice variant of p63) to be more specific for squamous differentiation than anti-p63. We compare the specificity of p40 and p63 in distinguishing AFX from SpSCC.

Design: 80 cases of primary cutaneous malignancies were selected, including 27 moderately (M-SCC) and 26 poorly differentiated SCCs (PD-SCC) and 27 AFXs, and immunohistochemical studies for p40 and p63 were performed. McNemar's test was used to compare sensitivity and specificity.

Results: 53 cases of SCC (27 M-SCC and 26 cases of PD-SCC) from 44 men and 7 women with a mean age of 69.6y (range: 50-87) and 27 cases of AFX from 21 men and 5 women with a mean age of 71.4y (range: 30-94) were studied. The results are summarized.

p40/p63 Expression in SCC and AFX

	M-SCC (n=27)	PD-SCC (n=26)	AFX (n=27)
p63 in >25% tumor nuclei	27/27 (100%)	24/26 (92.3%)	1/27 (3.7%)
p63 in 5-25% tumor nuclei	None	2/26 (7.7%)	7/27 (25.9%)
Cumulative p63 Expression	27/27 (100%)	26/26 (100%)	8/27 (29.6%)
p40 in >25% tumor nuclei	27/27 (100%)	21/26 (80.8%)	0/27 (0%)
p40 in 5-25% tumor nuclei	None	3/26 (11.5%)	0/27 (0%)
Cumulative p40 Expression	27/27 (100%)	24/26 (92.3%)	0/27 (0%)

The overall sensitivity/specificity of p40 and p63 for the distinction of PD-SCC and AFX was 92/100 and 100/70, respectively. Comparisons of the sensitivity and specificity between p40 and p63 were performed applying a cutoff of greater than 0% positivity in the tumor nuclei. For sensitivity, there was no difference between anti-p40 and anti-p63 (p=0.48); however, for specificity, p40 performed significantly better than p63 (p=0.01).

Conclusions: The application of p40 immunohistochemistry to evaluate spindle cell malignancies of the skin improves diagnostic specificity in distinguishing pSCC from AFX and thus can be helpful in the differential diagnosis of cutaneous spindle cell neoplasms.

541 Immunohistochemistry for Histone H3K79me3T80ph Is Superior to PHH3 in the Detection of Mitotic Chromatin and G2 Positive Nuclei in Merkel Cell Carcinoma

SA Henderson, MT Tetzlaff, P Pattanaprichakul, PS Fox, CA Torres-Cabala, RL Bassett, VG Prieto, HW Richards, JL Curry. MDACC, Houston, TX; Mahidol University, Bangkok, Thailand; Berkeley Lab, Berkeley, CA.

Background: Mitotic chromatin count (MC) is an important histologic parameter in tumor classification and prognosis in human malignancy. Chromatin condensation during G2/M phase of the cell cycle is an essential component of mitosis. An important regulator of chromatin condensation is posttranslational modification of histones. PHH3 (Ser 10) and H3K79me3T80ph (K79) are essential modifications for mitosis and share similar temporal regulation: both appear in G2 thru M phase of the cell cycle. PHH3 has been shown to facilitate MC in several tumors (e.g. melanoma, astrocytomas, prostate cancer) and K79 identify a subset of primary invasive melanomas with risk for metastasis. We compared the immunodetection of mitotic figures (MF) and G2 positive (+) tumor nuclei with anti-PHH3 and anti-K79 along with the proliferative marker Ki-67 in Merkel cell carcinoma (MCC) and correlated these findings with clinical outcome.

Design: 21 cases of MCC were selected from our archives (2000-2010) with known clinical outcome and performed immunohistochemistry for Ki-67, PHH3 and K79. MC (either by H&E or immunodetection) and/or number of positive Ki-67 tumor cells or G2+ tumor nuclei (identified as speckled nuclear labeling in an intact nucleus) for PHH3 or K79 was determined by "hot spot" approach in a mm2 of tumor (4.5 hpf). Comparison of MC and G2+ tumor nuclei was made with paired Student's t-test. Univariate Cox proportional hazards regression models were performed to examine different covariates of interest with survival.

Results: MCC were from 17 men and 4 women with a mean age of 68 years (range:56-83). The mean MC and G2+ tumor nuclei by H&E, PHH3 and K79 are in table 1. Compared with PHH3, K79 detected a higher number of MF (*) and G2+ tumor nuclei (†). Furthermore, the combined MC together with G2+ tumor nuclei detected with K79 was a stronger predictor of impaired survival than PHH3 and Ki-67 in patients with MCC (p=0.035) with 1.72% increased risk of death for each unit increase. There was mild evidence of an association with impaired survival for PHH3 and Ki-67.

Table 1	H&E: Mean (SD)	PHH3: Mean (SD)	K79: Mean (SD)
MC/mm2	12 (7.46)	39 (26.62)	58 (37.90)*
G2+/mm2	n/a	7 (5.21)	10 (5.84)†

SD=std. dev, *(p<0.0001), †(p<0.0052)

Conclusions: Immunodetection of MF and G2+ tumor nuclei in MCC with K79 results in higher values than PHH3 and correlates with increased risk of death. Thus immunodetection of both MF and G2+ tumor nuclei with anti-K79 appear to have biologic significance in MCC.

542 Differential Expression of T-Bet in Progression of Mycosis Fungoides and Its Histological Mimic, Pityriasis Lichenoides Chronica

A Hsi, A Kelley, A Musiek, A Schaffer. Washington University School of Medicine, St. Louis, MO.

Background: Mycosis fungoides (MF), the most common type of cutaneous T cell lymphoma, progresses through different stages from a few patches and plaques in the early stage to a more severe tumor stage. The early stage of mycosis fungoides can resemble a non-specific rash and can be difficult to differentiate from other benign dermatoses clinically and histologically. There have been studies suggesting that cutaneous lesions in early stage MF are characterized by a T-helper-1 (Th1) cytokine profile, while the profiles for tumor stage MF and benign mimickers of early stage MF have not been clearly delineated. In this study, we examined the expressions of T-bet in different stages of MF, as well as the use of T-bet in differentiating between early stage MF and pityriasis lichenoides chronica (PLC), which share similar histological appearances. T-bet is a transcription factor required for Th1 development from naive T-helper precursor cells, and thus serves as a marker for the presence of Th1 cells. Knowledge of the nature of the T-cell infiltrates may be important in better diagnosing and understanding these diseases.

Design: Sixteen cases of MF (9 patch/plaque, 7 tumor-stage) and 6 cases of PLC were examined for IHC expression of T-bet. The lymphocytes were evaluated for nuclear staining pattern. The percentage of positive cells is quantified as follows: ≤10% = negative, 11-30% = weak; 31-50% = moderate, and >50% = strong expression.

Results: 8/9 (89%) of patch/plaque stage MF cases showed moderate and strong T-bet expressions. In contrast, only 1/6 (17%) of PLC showed a moderate expression, with 6/7 (83%) of cases showing negative to weak expressions of T-bet. 4/7 (57%) of tumor stage MF were negative for T-bet, while 3/7 (43%) showed moderate and strong expressions.

	Percentage of Lymphoid Infiltrate Positive (%)			
	0-10%	11-30%	31-50%	>50%
Patch/plaque stage	0/9 (0)	1/9 (11)	2/9 (22)	6/9 (67)
Tumor stage	4/7 (57)	0/7 (0)	1/7 (14)	2/7 (29)
PLC	2/6 (33)	3/6 (50)	1/6 (17)	0/6 (0)

Conclusions: The majority of early stage MF cases were strongly positive for T-bet, suggesting a predominance of Th1 cells in the infiltrate. In contrast, >50% of tumor and >80% of PLC showed negative or weak expressions of T-bet, indicating an absence or decrease in Th1 cells compared to early stage MF. In addition, our preliminary findings suggest that T-bet may be a reliable supportive marker in differentiating early stage MF from PLC in morphologically challenging cases. These results will be further validated in a larger number of cases.

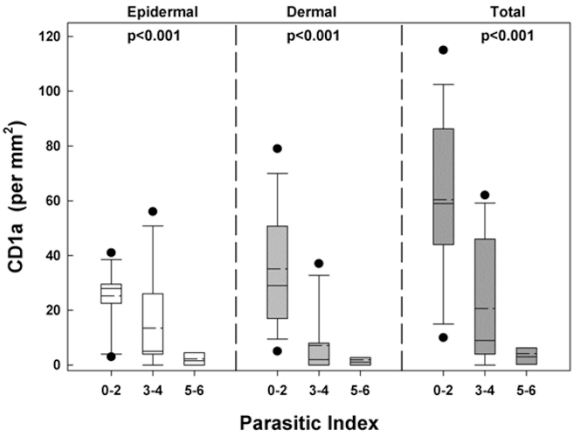
543 Immune Microenvironment in Cutaneous Leishmaniasis: A Potential Target for Immunotherapy

M Jabbour, K Charafeddine, G Issa, H Khalifeh, R Habib, I Khalifeh. American University of Beirut, Beirut, Lebanon.

Background: Cutaneous leishmaniasis (CL) has spread to non-endemic regions, propelling the recent interest into better understanding of this neglected infection. Downregulation of the CD1a receptor on Langerhans cells (LCs) has been described in various cutaneous infections. In this study, the immune response across different Ridley patterns (RPs) and parasitic indices (PI) is outlined in CL.

Design: Skin punch biopsies from the interface of normal and lesional CL were collected from 33 patients with molecularly confirmed *Leishmania Tropica* infection. RPs (2-5) were assessed for various clinicopathologic features including age, gender, disease duration, PI and constituents of the inflammatory infiltrate. CD1a, CD68, CD3, CD4, CD8, CD20 and CD138 stains were performed on normal skin, CL biopsies and leishmania promastigote identified on cytospin/cell block preparations collected from culture. CD1a was quantified per mm² in the epidermis and dermis. The remaining stains were graded according to a 4-teired grading system [0 (0 – 4%); 1 (5 – 24%); 2 (25 – 49%); 3 (50 – 74%) and 4 (75 – 100%)].

Results: Total CD1a expression significantly decreased (14-fold) from PI (0-2) to PI (5-6); (ρ <0.001).



CD1a expression in the epidermis was at least 5-fold lower than normal skin (58 vs. 400 cells/mm²) and demonstrated epidermal to dermal migration, inversely correlating with the PI (33 vs. 0 cells/mm² in the dermis). The amastigotes revealed diffuse and strong staining with CD1a and CD68 antibodies in all cases however, promastigotes were negative. The major inflammatory infiltrate consisted of macrophages and CD3+CD4-CD8- T-regulatory lymphocytes in all RPs. Interestingly, the parasitic-laden macrophages were ringed by T-regulatory cells. Apart from CD1a, there was no significant difference in inflammatory markers between the various RPs and PIs. Disease duration did not correlate with RP.

Conclusions: Significant decrease in CD1a expression is postulated by two mechanisms; either via direct CD1a receptor uptake by amastigotes and/or antigenic stimulation of T-regulatory cells causing inhibition of CD1a LCs. CD1a/CD68 coated amastigotes may represent potential prophylactic and therapeutic targets in CL.

544 DOG1 Expression in Cylindroma

M Jacob, RA Robinson. University of Iowa, Iowa City, IA.

Background: DOG1 is a calcium-activated chloride channel first found to be differentially expressed in gastrointestinal stromal tumors. More recently, its expression has been reported in salivary gland tissue, normal and neoplastic. As there is some basic functional and morphologic similarities between normal salivary and eccrine glands as well as between cylindroma and some salivary gland tumors, we wished to determine if DOG1 was expressed in cylindroma and eccrine glands.

Design: We investigated 25 cases of cylindroma (CYL) and the surrounding normal eccrine glands in the excised tissue. Formalin fixed paraffin embedded sections were deparaffinized and pre-treated with heat induced epitope retrieval. The antibody DOG1 (NCL-L-DOG1, Leica Biosystems) was used at a dilution of 1:100. Staining was regarded as none if no staining was present, focal if 1-25% of the cells expressed staining, moderate for 25-50% and diffuse for >50%. Intensity for staining was categorized from 1(least) to 3(most) intense. Luminal or abluminal location of staining was noted. Cellular localization was further evaluated for cytoplasmic or membranous staining.

Results: 17/25 of CYL showed staining; 10/17 focal, 5/17 moderate and 2/17 diffuse. When staining was present, luminal staining was seen in 17/17 cases and abluminal in 12/17. All positive CYL cells showed membranous staining and 12/17 also showed cytoplasmic staining. Staining intensity was 1+ (13/17), 2+(3/17) and 3+(1/17). There was no staining of any cells associated with production of basement membrane like material. Cells rimming gland-like lumina never exhibited staining. All eccrine glands stained with 2+ to 3+ staining intensity of intercellular canaliculi and 2+ staining in the apical portions of the glandular unit, all of which were membranous with no cytoplasmic staining. Eccrine glands showed no abluminal staining.

Conclusions: CYL expresses DOG1 in a membranous and cytoplasmic pattern, with both luminal and abluminal portions of tumor nests. Eccrine glands show very strong intercellular canalicular and gland luminal staining in a membranous pattern. No staining of myoepithelial (abluminal) cells was present in the eccrine glands. The lack of abluminal staining in normal eccrine glands contrasted to the frequent abluminal staining in CYL. It is unclear why there is an expression difference of DOG1 between normal and neoplastic myoepithelial cells. Overall the findings of this study may lead to better understanding of the role DOG1 plays in normal eccrine gland function and further define the biology of CYL as well provide insights into potential therapy.

545 Melanoma Genotypes Using Targeted Next Generation Sequencing

MS Jacobs, JD Peterson, F de Abreu, TL Gallagher, CI Amos, MS Ernstoff, GJ Tsongalis, JB Brennick. Dartmouth-Hitchcock Medical Center, Lebanon, NH; Geisel School of Medicine at Dartmouth, Hanover, NH.

Background: Advanced stage malignant melanoma often responds poorly to conventional chemotherapy and radiation therapy, with five-year survival rates below 10%. New therapeutic approaches are based upon a growing understanding of the molecular abnormalities in melanoma. Several pathways have been implicated, including the RAS-RAF-MEK-MAPK pathway with activating mutations in BRAF and NRAS and the PI3K/AKT pathway with activating mutations in NRAS or inactivation of PTEN. Here, we demonstrate the feasibility of a next generation sequencing assay, which targets hotspots in 50 cancer genes, to assess melanoma genotypes that may influence therapeutic selection and response.

Design: DNA was extracted from 10 unstained tissue sections. Percent tumor content was assessed by a pathologist. Nine melanoma cases including 1 primary melanoma and 8 metastatic melanomas (liver, 4; lymph node, 2; skin, 2) were submitted for clinical sequencing. Up to 10ng of DNA was used to create barcoded libraries which were multiplexed on Ion Torrent 318 chips and sequenced with the AmpliSeq Cancer Hotspot Panel v2. Variants were identified using the Ion Torrent Variant Caller Plugin (v.3.6.59049). Variant annotation and functional predictions were performed using Golden Helix SVS (v.7.7.3).

Results: Of the 9 cases analyzed, 2 showed wild type sequences for hotspots in 50 cancer genes. BRAFV600E mutations were present in 3 cases and NRAS mutations in 4 cases. We identified a CTNNB1 (c.110C>T) mutation in a case which also harbored an NRAS mutation. Additionally, a CDKN2A (c.389T>A) mutation was present as a second mutation in a case with a BRAF mutation.

Conclusions: It is increasingly clear that melanoma is a heterogeneous disease. Activating mutations in BRAF are associated with a good prognosis and may respond to selective BRAF inhibitors. Activating mutations in NRAS are associated with a worse prognosis but may respond to MEK inhibitors. Our data highlights a less frequently observed mutation in the CTNNB1 oncogene which results in stabilization of the β-catenin protein. Currently, no direct anti-β-catenin therapies are available. Finally, the CDKN2A gene locus encodes 3 tumor suppressors, including p16/INK4a and p14/ARF, which play key roles in the Rb and p53 tumor suppressor pathways, respectively. This CDKN2A pathway may represent an ideal target for future therapies given its role in two major cell cycle regulatory pathways.

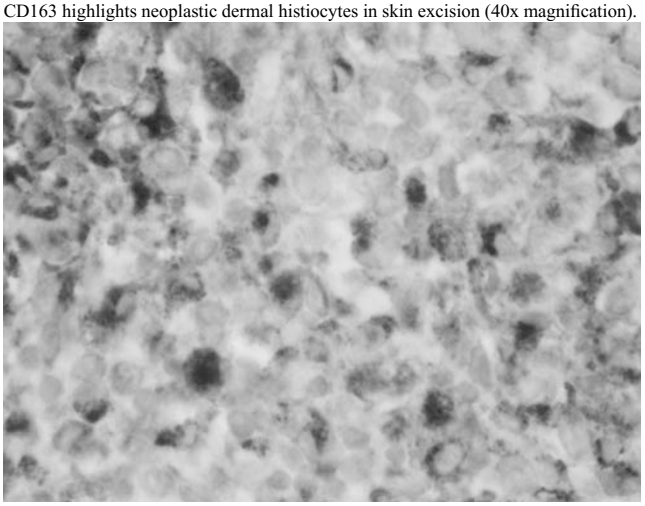
546 Histiocytic Sarcoma: A Report of Five Cases with Primary Cutaneous Involvement

N Kazi, C Magro. Columbia University Medical Center, New York; Weill Medical College of Cornell University, New York.

Background: Histiocytic sarcoma is an extremely rare tumor of true histiocytic origin. Extracutaneous histiocytic sarcoma follows an aggressive clinical course. We present 5 cases of primary cutaneous histiocytic sarcoma outlining its clinical and light microscopic features.

Design: Cases of primary cutaneous histiocytic sarcoma were identified using a natural language search from the dermatopathology data base of Cornell University.

Results: There was a male predominance in the patients (4 males and 1 female) ranging in age from 33 years to 92 years (mean age of 58 years), who presented with solitary nodules (in four) and a diffuse eruption in one. The face was involved in three. In one patient, there was a history of acute B cell lymphocytic leukemia; another patient had chronic myeloproliferative disorder. In the remaining patients, there was no significant medical history. Biopsies showed an extensive nonepitheliotropic, highly atypical large cell histiocytoid appearing infiltrate within the dermis. In all five cases, the infiltrate showed extensive staining for common leukocyte antigen, CD4, CD14, CD68, CD163, CD2, lysozyme, and CD4. In none of the cases was there evidence of extracutaneous dissemination. The treatment in 4 of the cases was re-excision; there was no evidence of disease progression. Figure 1: Representative immunohistochemical stain with CD163 highlights neoplastic dermal histiocytes in skin excision (40x magnification).



Conclusions: Histiocytic sarcoma must be differentiated from malignant fibrous histiocytoma and atypical fibroxanthoma. Staining for common leukocyte antigen and CD163 are the most reliable markers allowing this distinction. Patients who present with primary involvement of the skin have a favorable outcome. Its existence with B cell and myeloid dyscrasias suggests a potential B cell or myeloid line of ontogeny for these neoplasms.

547 Keratoderma-Like T Cell Dyscrasia: A Report of 13 Cases and Its Distinction from Mycosis Fungoides Plantaris/Palmaris

N Kazi, R Abraham, C Magro. Columbia University Medical Center, New York, NY; Weill Medical College of Cornell University, New York, NY.

Background: Atypical epitheliotropic T cell lymphocytic infiltrates are commonly encountered in routine and consultative dermatopathology practices and not all represent mycosis fungoides. An important and common category are the cutaneous T cell dyscrasias such as pityriasis lichenoindes, pigmented purpuric dermatosis, and alopecia mucinosa.

Design: Cases of cutaneous lymphoid dyscrasia localized to the palms and soles were identified using a natural language search from the dermatopathology data base of Cornell University.

Results: Thirteen cases were identified in which patients presented with a palmar and/or plantar keratoderma without other sites of cutaneous involvement. The lesions were persistent and largely resistant to topical steroids. Biopsies show a variably dense superficial angiocentric CD4 or CD8 dominant lymphocytic infiltrate accompanied by a nondestructive pattern of epitheliotropism. Low grade cerebriform atypia along with variable diminution in the expression of CD7 and CD62L was noted. In two cases, statins were likely causative. The treatments included intramuscular steroids and methotrexate. There were no cases of disease progression to mycosis fungoides.

Conclusions: The spectrum of cutaneous lymphoid dyscrasias should be expanded to cases showing an acral based localization manifesting as keratoderma. These cases are to be distinguished from mycosis fungoides palmaris et plantaris. As with other forms of cutaneous lymphoid dyscrasia, the lesions tend to be persistent but have an indolent course.

548 Implementation of a National Plan in Argentina to Detect Braf V600 Mutation-Positive Metastatic Melanoma

MC Latorre, M Gutierrez, HJ Garcia-Rivello, B Livellara, GC Lozano, C Maldonado, VI Volonteri, P De La Iglesia, D Aylagas, G Garritano, M Carrizo, EM Mocetti, F Moffa. Productos Roche, Tigre, Provincia de Buenos Aires, Argentina; Laboratorio Stamboulian, Buenos Aires, Argentina; Hospital Italiano de Buenos Aires, Buenos Aires, Argentina; San Isidro Patologia, San Isidro, Buenos Aires, Argentina.

Background: BRAF is a proto-oncogene of the RAS RAF MEK ERK signaling pathway. BRAF mutations are frequent in melanoma (MM), thyroid carcinoma and other neoplasias. BRAF V600E is the most prevalent mutation accounting for over 80% of all allelic variants. Most mutations increase BRAF kinase function, thus providing constitutive oncogenic signaling through MEK. Mutant BRAF is the target of vemurafenib, recently authorized for treating locally advanced or metastatic melanoma (mM). We present the preliminary results of screened tumor samples evaluated for BRAF mutations in two reference centers in Argentina.

Design: Primary (n=147) and metastatic (n=176) melanomas from 323 patients were sent for screening for BRAF V600 mutations using real-time PCR (COBAS 4800, Roche). The detection of BRAF V600 mutations was performed in DNA isolated from macrodissected formalin-fixed, paraffin-embedded melanoma tissue.

Results: Histopathological data were correlated with the BRAF mutational status. Four biopsies were rejected due to insufficient sample. Of the 319 samples analyzed for BRAF V600 mutations, 174 (54,6%) were mM and 145 (45,4%) were primary lesions: nodular MM (n: 54, 49,5%), superficial spreading M (n:26, 23,9%), lentigo maligna MM (n: 3, 2,8%), acral lentiginous MM (n:10, 11%), miscellaneous types (n:12, 9%) and not classified (n:40, 27,5%). The most frequent metastatic site were the lymph nodes (36,3%), skin/ soft tissue (28,7%), brain (7,5%), gastrointestinal tract (6,6%), lungs (6,2%) and liver (6%). BRAF V600 mutation was found in 139 (43,6%) of the 319 samples analyzed. Four cases were Indeterminate because of PCR inhibition (1,2%). Nodular MM showed a higher incidence of positive mutations (36/54 cases, 66,67%). Superficial spreading and acral lentiginous MM were positive in 34.6% (9/26) and 20% (2/10) of the cases, respectively. The stastical analysis showed significant differences in distribution of histological subtypes by BRAF status; nodular MM subtype represents 76,6% of all mutated primary MM.

Conclusions: The preliminary results of the Argentine plan to detect BRAF V600 mutation-positive melanomas are comparable to those reported in the international literature. It is noteworthy the high incidence of BRAF positive mutations in nodular melanomas, as well as the relatively high frequency observed in the subgroup of acral lentiginous melanomas.

549 Subcutaneous Panniculitis-Like T-Cell Lymphoma and Lupus Erythematosus Panniculitis: A Challenging and Critical Distinction

RE LeBlanc, J Kim. Stanford University School of Medicine, Stanford, CA.

Background: Occasionally, the distinction between subcutaneous panniculitis-like T-cell lymphoma (SPTCL) and lupus erythematosus panniculitis (LEP) is challenging. SPTCL offers a worse prognosis and can progresses to hemophagocytic lymphohistiocytosis (HLH), which may be fatal. While clonality aids in this distinction, the finding is not readily demonstrable in every case of lymphoma and there are reports of clonality in patients with longstanding LEP. The scope of this study is to further distinguish histologic features that separate these rare entities and predict progression to HLH.

Design: Slides were reviewed from 27 patients with diagnoses of SPTCL (14), closely related PCGDTCL (6), and LEP (7). Histomorphologic features (hyaline lipomembranous necrosis, vasculitis and fibrinoid necrosis, macrophages engulfing karyorrhectic debris, classic adipocyte rimming, lymphocyte atypia, plasma cell distribution, adnexal accentuation, dermal interstitial mucin, and germinal center follicles) and quantitative assessment of Ki-67 and CCL5 positive cells and CD123 positive cell clusters were correlated with clinical data.

Results: Over a mean follow-up period of 3.4 years for SPTCL, 3.0 years for LEP, and 1.6 years for PCGDTCL, 7 (50%) patients with SPTCL and 1 (17%) patient with PCGDTCL developed HLH. One patient with PCGDTCL exhibited an indolent course with spontaneous resolution of lesions. All but one SPTCL case showed > 50% Ki-67 staining, whereas all but one LEP case showed < 25% staining. CCL5 revealed > 50% staining in most lymphomas while all LEP cases revealed < 50%. CD123 staining was similar between groups, but the absence of dendritic cells was seen only in lymphomas. Present in all LEP cases were plasma cell infiltrates and lipomembranous necrosis, which was observed just focally in 7 (50%) of SPTCL cases. Lymphocyte atypia was seen exclusively in lymphoma and adipocyte rimming was exclusively present in 10

(71%) of SPTCL cases. None of the findings predicted progression to HLH, although SPTCL cases that progressed had extensive adipocyte rimming.
Conclusions: The prevalence of HLH among patients with SPTCL at our institution was higher than the generally reported prevalence. Our data further substantiate reports of an indolent PCGDTCL subtype. Ki-67 and CCL5 immunostains most effectively distinguished LEP from SPTCL, while the most informative morphologic features entailed lipomembranous necrosis, plasma cell density, adipocyte rimming, and lymphocyte atypia. Additional data are needed to predict which subsets of SPTCL and PCGDTCL patients progress to HLH.

550 Tumor Size Correlates with Survival in Melanomas Associated with Conventional or Cellular Blue Nevi or Displaying Blue Nevus-Like Features (So Called Malignant Blue Nevus)

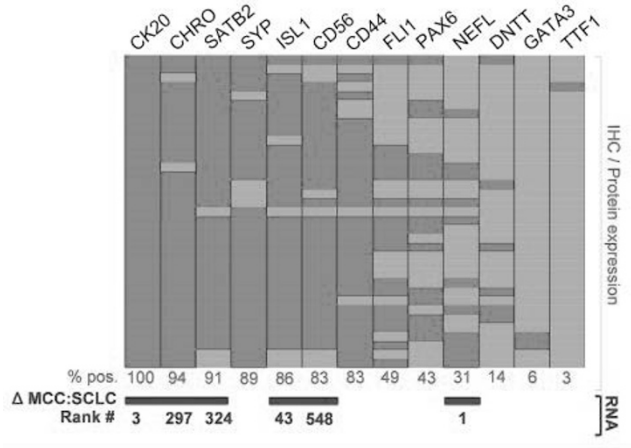
S Loghavi, C Torres-Cabala, J Curry, D Ivan, R Bassett, V Prieto, M Tetzlaff. The University of Texas MD Anderson Cancer Center, Houston, TX.
Background: Melanomas associated with conventional or cellular blue nevi are uncommon lesions posing unique diagnostic challenges. Given their usual intradermal location without a prominent intraepidermal component, it remains controversial whether conventional parameters predictive of outcome in standard cutaneous melanomas apply to these lesions.
Design: Twenty-four cases of primary invasive melanoma associated with a benign blue nevus component were reviewed from the past 9 years at a tertiary cancer center. Overall survival (OS), recurrence-free survival (RFS), time to local recurrence (TLR), and time to distant recurrence (TDR) were recorded. Cox proportional hazards regression models were fit to model the association between each survival parameter and clinical and tumor covariates of interest. No adjustment was made for the multiplicity of testing.
Results: 24 lesions were retrieved from 15 women and 9 men with a mean age of 50 y (range: 20-85). These melanomas most often develop on the head and neck (n=12; 50%) or trunk (n=9; 38%). Histologically, these tumors typically arise in the dermis and/or subcutaneous tissue without an intraepithelial component (96%). The mean *tumor size* (defined as the single greatest tumor dimension either Breslow thickness or largest diameter) was 20.9 mm (range: 0.6-130 mm). The mean mitotic count was 6.5/mm2 (range: 2-30). Perineural invasion was common (n=9; 38%). Follow-up was available for 21 cases (range: 0.4 to 11.6 years with a median of 2.1 years). The median OS, RFS, TLR, and TDR were 5.2, 0.7, 2.6, and 1.6 y, respectively. Cox regression analyses demonstrated only *tumor size* to correlate with clinical outcome: there was a significant association between tumor size and RFS (HR=1.02 per mm; p=0.04) and reduced TDR (HR=1.03 per mm; p=0.02) with a similar trend towards reduced TLR (HR=1.02 per mm; p=0.07). No other parameter (age, anatomic location, mitotic figures, lymphovascular or perineural invasion, or type of associated blue nevus) emerged as significant.
Conclusions: Melanomas associated with blue nevi are rare with an anatomic predilection for the head and neck region. Here, we show that tumor size correlates with reduced recurrence-free survival and time to distant recurrence.

551 Pediatric Conventional Melanoma Shares Similar Genomic Features with Its Adult Counterpart

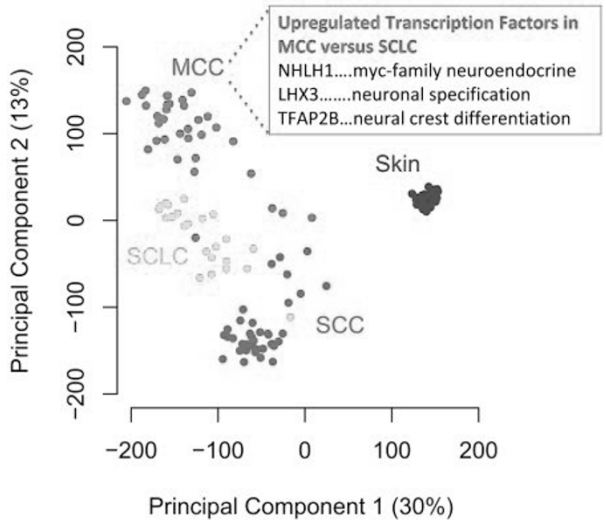
C Lu, P Nagahawatte, J Easton, Z Liu, S Pounds, A Pappo, A Bahrami. Washington University School of Medicine, St. Louis, MS; St. Jude Children's Research Hospital, Memphis, TN; For the St. Jude Children's Research Hospital-Washington University, Memphis, TN.
Background: Melanoma in patients <20 years of age is rare but its incidence continues to rise at a rate of 2% per year. Over the past decade, the genomic landscape of adult melanoma has been well characterized and therapeutically exploited whereas the molecular biology of pediatric melanoma remains largely unknown.
Design: Samples from 23 pediatric patients with non-familial conventional (15 *de novo*; 3 arising in congenital nevi) and Spitzoid (n=5) melanoma were analyzed using whole genome sequencing (n=5), whole exome sequencing (n=10), molecular inversion probe assay (n=19), and targeted sequencing of *BRAF* (n=23), *NRAS* (n=20), and *TERT* promoter (n=20).
Results: Conventional-type melanomas exhibited multiple copy number changes with common regions of alterations similar to adult melanoma. The oncogenic driver was a mutation at *BRAF* V600 in 87% of the *de novo* conventional melanomas and a mutation at *NRAS* Q61 in the 3 melanomas arising within congenital nevi. All *de novo* conventional melanomas had a high mutation rate and an enrichment of UV-induced mutations. Excluding an acral melanoma, all *de novo* conventional melanomas carried a *TERT* promoter UV-signature mutation, as did the only Spitzoid melanoma with a fatal outcome.
Conclusions: Genomic profiling supported a molecular basis for the contribution of UV light in the pathogenesis of pediatric melanoma. Pediatric conventional and adult melanoma share similar genomic features, suggesting that emerging therapies for genotype specific adult melanomas are applicable to conventional melanomas affecting the pediatric population.

552 Integration between Immunohistochemistry (IHC) and mRNA Expression Patterns Reveals Novel Biomarkers for Merkel Cell Carcinoma

M McFall, S Mohanty, A Conley, S Bhele, X Yuan, M Amin, B Knudsen, D Dhall, B Balzer. Cedars-Sinai Medical Center, Los Angeles, CA.
Background: Merkel Cell Carcinoma (MCC) is an aggressive cutaneous neuroendocrine (NE) neoplasm morphologically similar to small cell carcinoma. A limited IHC panel has been reported for this differentiation. We analyze a broad IHC panel in MCC and compare RNA expression datasets of MCC and Small Cell Lung Carcinoma (SCLC) to identify novel biomarkers.
Design: A TMA was constructed from 35 archival MCC cases and stained with synaptophysin (SYP), chromogranin (CHGA), CD56, CK20, neurofilament (NEFL), SATB2, ISL1, DNTT, FLI1, PAX6, PAX5, GATA3 and TTF1 antibodies and scored as +/-. Using a principal component analysis (PCA), RNA expression was examined in publically available microarray datasets (30 MCC, 4 MCC cell lines, 36 squamous cell carcinoma (SCC), 22 SCLC and 64 normal skin). Genes differentially expressed between MCC and SCLC were selected above a 4-fold difference in expression and a false discovery rate (FDR) < 0.01.
Results: The IHC expression pattern is shown in Figure 1 (red=+ cases; grey=- cases). With the exception of PAX6, SATB2 and GATA3, markers in the panel have previously been analyzed in MCC. Genes upregulated in MCC vs. SCLC (n=756, blue bar) are ranked according to the fold change and the rank number is listed.



A principle components analysis (PCA) of global mRNA expression reveals well separated clusters of MCC, SCLC, SCC and skin (Figure 2). The first PC contains 30% and the second PC 13% of variability in the gene set. Three NE-associated transcription factors (NHLH1, LHX3 and TFAP2B) are in the top 20 differentially expressed genes.



Conclusions: SYP, CHGA, CD56, CK20, SATB2 and ISL1 demonstrate high frequency of expression in MCC; SATB2, GATA3 and PAX6 are novel markers for subsets of MCC; PCA of mRNA expression demonstrates a clear separation between MCC and SCLC; NHLH1, LHX3 and TFAP2B are potential markers to distinguish MCC from SCLC by in situ hybridization.

553 Diagnostic Utility of an Expanded Panel of Novel and Well-Established Immunohistochemical (IHC) Markers to Differentiate Merkel Cell Carcinoma from High-Grade Non-Merkel Cell Neuroendocrine Carcinomas

MJ McFall, SK Mohanty, G Liles, H Lugo, F Chung, B Knudsen, X Yuan, MB Amin, D Dhall, B Balzer. Cedars-Sinai Medical Center, Los Angeles, CA.
Background: When the primary site is unknown, differentiation of Merkel cell carcinoma (MCC) from high-grade non-Merkel cell neuroendocrine carcinomas (HGNMNEC) can be morphologically challenging. This distinction has significant therapeutic implications. Without clinical and radiologic information, IHC is used to arrive at the final diagnosis. Although limited IHC panels addressing this question have been reported, an extensive evaluation is lacking. We sought to expand the IHC panel differentiating MCC from HGNMNEC by testing the utility of a variety of neuroendocrine markers(synaptophysin, SYP and chromogranin, CHGA), transcription factors(ISL1, PAX8, PAX6, PAX5, TTF1, CDX2, SATB2, Tdt, FLI1, ERG, GATA3),

adhesion molecules(E-cadherin, CD44, CD56), microtubule-related intermediate filaments(CK20 and neurofilament, NF), antiapoptotic marker(bcl2), stem cell growth factor receptor(CD117) and transmembrane glycoprotein(CD99).

Design: 35 MCC and 42 HGNMNEC cases of which 23 were small cell carcinoma(SCC) (21 lung, 11 bladder, 5 gastroenteropancreatic,GEP and 5 gynecologic,GYN) were selected and a paraffin TMA constructed. The above-mentioned IHC markers were performed and evaluated on a scale from 0-3.

Results: The mean expression of CHGA, SYP and CD56 for MCC and HGNMNEC are 94%, 89%, and 83% and 55%, 93% and 83%, respectively.

Table 1. Differences in IHC expression of MCC, HGNMNEC and SCC.

	CK20	Tdt	NF	TTF1	CDX2	PAX8
MCC(n=35)	35(100%)	29(83%)	18(51%)	1(3%)	0	0
HGNMNEC(n=42)	4(10%)	0	0	22(52%)	12(29%)	11(26%)
SCC(n=23)	4(17%)	0	0	10(43%)	8(35%)	7(30%)

Table 2. Differences in the expression pattern by anatomic location of IHC Markers HGNMNEC.

	CK20	Tdt	NF	TTF1	CDX2	PAX8	GATA3
Lung(n=21)	1(5%)	0	0	18(86%)	4(19%)	5(24%)	0
GEP(n=5)	1(20%)	0	0	2(40%)	3(60%)	2(40%)	0
Bladder(n=11)	1(9%)	0	0	1(9%)	4(36%)	4(36%)	5(45%)
GYN(n=5)	1(20%)	0	0	1(20%)	2(40%)	0	0

In MCC, Tdt and NF are 100% specific and 83% and 51% sensitive, respectively. CK20 shows 100% sensitivity and 90% specificity for MCC. TTF1 is 86% sensitive and 93% specific for lung but is present in 19% of extrapulmonary tumors. CDX2 is 60% sensitive and 86% specific for GEP but is observed in 27% of non-GEP HGNMNEC.

Conclusions: A selective IHC panel consisting of CK20, Tdt and NF(supporting MCC), TTF1(suggesting pulmonary origin), CDX2 and PAX8(suggesting GEP origin) and GATA3(suggesting bladder origin) has diagnostic utility in differentiating MCC from HGNMNEC.

554 Does Immunohistochemistry Add to Morphology in Differentiating Trichoepithelioma, Desmoplastic Trichoepithelioma, Morpheaform Basal Cell Carcinoma and Microcystic Adnexal Carcinoma?

MJ McFall, SK Mohanty, A Usmani, JM Wu, D Frishberg. Cedars-Sinai Medical Center, Los Angeles, CA; Kaiser Permanente Medical Center, Los Angeles, CA.

Background: The distinction among cutaneous basaloid neoplasms such as trichoepithelioma (TE), desmoplastic trichoepithelioma (DTE), morpheaform basal cell carcinoma (MBCC) and microcystic adnexal carcinoma (MAC) can be difficult, especially in superficial biopsies. Accurate characterization is important for appropriate management. While TE and DTE are benign neoplasms with indolent behavior, MBCC and MAC are typically locally aggressive. The expression of several recently described immunohistochemical (IHC) markers, including p40, IMP3 and ProEx C, has not been established in cutaneous neoplasms. We explored the potential utility of a broad IHC panel, including previously reported and novel markers to differentiate TE, DTE, MBCC and MAC.

Design: A total of 28 archival cases [TE (n=14), DTE (n=6), MBCC (n=6) and MAC (n=2)] were stained with 9 IHC markers: p40, IMP3, ProEx C, p16, CK20, Ki-67, androgen receptor (AR), D2-40 and beta-catenin. Tumors with greater than 5% immunoreactivity were scored as positive. Intensity was scored on a scale from 1+ to 3+. The pattern of positivity (nuclear (N), cytoplasmic (Cy), membranous (M), or in combination; peripheral (p) or central (c) distribution within lesion) was also recorded.

Results: CK20 (in contrast to prior studies) and IMP3 were negative in all cases. Likewise, with the exception of one case of TE, AR showed no immunoreactivity in all categories. No significant difference was observed in the expression of beta-catenin, p16, ProEx C and p40 between the four groups of cutaneous neoplasms. The mean Ki-67 labeling index for MBCC (8%) was slightly higher than DTE (3%). Interestingly, the proliferation index for TE (15%) was significantly higher than that of MBCC. Both cases of MAC (interpretation is somewhat limited by sample size) and 57% of TEs expressed D2-40; neither the MBCC nor DTE cases showed D2-40 immunoreactivity. Also, we confirm the previously published observation of scattered CK20 positive Merkel cells in the epidermis of all cases of DTE. Whereas, no Merkel cells are identified in MBCC and MAC cases.

Conclusions: With the exception of Ki-67, our IHC panel showed no significant added diagnostic utility of IHC in discriminating among TE, DTE, MBCC, and MAC.

555 Detection of Immunoglobulin Light Chain Restriction in Cutaneous B-Cell Lymphomas by Ultrasensitive Bright-Field mRNA In-Situ Hybridization

EC Minca, H Wang, K Wang, Z Wang, C Lanigan, SD Billings, Y Luo, X-J Ma, RR Tubbs. Cleveland Clinic, Cleveland, OH; Advanced Cell Diagnostics, Hayward, CA.

Background: Detection of immunoglobulin (Ig) light-chain restriction (LCR) is important in differentiating B-cell non-Hodgkin lymphoma (B-NHL) from benign lymphoid hyperplasia (BLH). Flow cytometry, commonly used to evaluate LCR, is impractical for cutaneous specimens. Immunohistochemistry (IHC) and conventional chromogenic in-situ hybridization (CISH) on formalin-fixed-paraffin-embedded (FFPE) tissue lack sufficient sensitivity to detect low-level Ig light-chain expression in B-NHL without plasmacytic differentiation. We assessed the utility of ultrasensitive bright-field mRNA-ISH (BRISH) for in-situ LCR detection in skin B-NHL.

Design: We tested FFPE skin biopsies and excisions from 29 patients with available PCR Ig clonality studies or FISH-confirmed genomic rearrangements: 18 clonal B-cell lesions (8 follicle center cell lymphoma, 5 marginal zone lymphoma, 3 large B-cell lymphoma, 2 other) and 11 non-clonal B-cell proliferations. Simultaneous detection of kappa and lambda light-chain mRNA was performed using two-color duplexed BRISH (RNAscope, Advanced Cell Diagnostics) and the scores were correlated with the PCR clonality / FISH status.

Results: BRISH-detected Ig light-chain mRNA expression was evaluable for 10/11 non-clonal B-cell proliferations and 18/19 clonal B-cell lesions. Expression was polytypic (non-restricted) in 9/10 non-clonal B-cell proliferations. LCR was present in 18/18 evaluable clonal B-cell lesions. In 4/5 marginal zone lymphomas, LCR was detected as strong monotypic mRNA expression in a B-cell subset, consistent with plasmacytic differentiation. The discordant result was an atypical B-cell non-clonal and T-cell clonal lymphoid proliferation, with a kappa LCR by BRISH. The overall concordance of BRISH with the PCR clonality studies was 96.3% in this series.

Conclusions: Ultrasensitive mRNA ISH can successfully detect LCR in B-NHL from FFPE skin specimens. Further studies are warranted to validate BRISH as a valuable ancillary diagnostic tool when LCR cannot be established by CISH or IHC.

556 Correlation of the Chemokine Receptor CXCR4 with BRAF Status and Histopathologic Prognosticators in Primary Cutaneous Melanoma

BC Mitchell, JK Feller, PP Aung, B Philip, S Yang, H-Y Park, M Mahalingam. Boston University School of Medicine, Boston, MA.

Background: A previous study on papillary thyroid carcinoma has shown that upregulation of the chemokine receptor CXCR4 involves defects in the Ras/Raf/MEK/ERK pathway, particularly relating to *BRAF*V600E. Given this and the increased prevalence of BRAFV600E in primary cutaneous melanoma (PCM) we hypothesize that elevated expression of CXCR4 can be correlated with the *BRAF* mutation status. A secondary aim was to correlate CXCR4 expression to established histopathologic prognosticators in PCM to ascertain its clinical utility as an independent predictor of an aggressive phenotype.

Design: Archival tissue samples with a diagnosis of primary cutaneous melanoma were obtained from the pathology files of the Skin Pathology Laboratory, Boston University School of Medicine, Boston, MA. A total of 87 cases were identified as meeting criteria for inclusion in the study. Molecular analysis for CXCR4 gene expression and BRAF exon 15 mutation status was performed using mRNA semi-quantitative RT-PCR and DNA Sanger sequencing respectively.

Results:

Criteria	Category	No. of samples	ΔCt +/- SD	*p*
BRAF Status	Mutant	22	5.69 +/- 1.40	0.00863
	Wild Type	65	6.60 +/- 1.70	
Thickness	<1mm	37	6.13 +/- 1.85	0.13429
	≥1mm	50	6.55 +/- 1.54	
Mitoses	Absent	31	6.80 +/- 2.12	0.07180
	Present	56	6.18 +/- 1.38	
Host Response	Absent	67	6.55 +/- 1.67	0.00028
	Present	19	5.39 +/- 1.01	
Ulceration	Absent	70	6.42 +/- 1.75	0.21625
	Present	15	6.10 +/- 1.35	
Regression	Absent	61	6.44 +/- 1.69	0.26056
	Present	24	6.18 +/- 1.70	
Vascular Invasion	Absent	85	6.36 +/- 1.68	0.00007
	Present	1	5.63 +/- 0.00	

Our data indicate a statistically significant correlation between elevated CXCR4 expression (low ΔCt value) and presence of the following in primary cutaneous melanoma: BRAF mutation, a brisk host response and vascular invasion.

Conclusions: These findings suggest that CXCR4 expression may be of utility as a biomarker for recruiting melanoma patients for immunotherapy. The presence of vascular invasion in only one case of PCM in our cohort challenges the relationship between this and CXCR4 expression despite a significant *p* value.

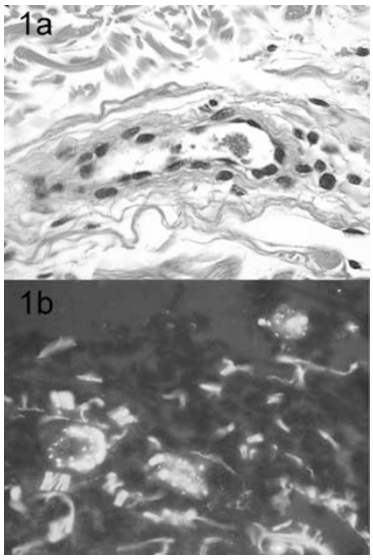
557 The Role of Skin Biopsy in the Diagnosis of Atypical Hemolytic Uremic Syndrome

S Momtahen, CM Magro. Weill Medical College of Cornell University, New York, NY.

Background: Atypical hemolytic uremic syndrome (atypical HUS) is one of the prototypic thrombotic microangiopathy syndromes with characteristic renal, liver and central nervous system involvement. Genetic mutations in complement can predispose to various diseases including atypical HUS. Among the mutations that have been implicated in atypical HUS are Factor H, Factor 1, CD46, C3, Factor B, and thrombomodulin. Although the skin is rarely involved in atypical HUS, we postulated that a biopsy of normal skin could reveal evidence of systemic endothelial based complement activation.

Design: Biopsies of normal skin were procured from the arm of seven patients with classic features of atypical HUS. Twelve additional biopsies were obtained from patients who did not fulfill diagnostic clinical criteria of atypical HUS. The samples were first examined by routine H and E. Immunofluroescent and immunohistochemical studies on fresh and formalin fixed tissue were also conducted to assess for C5b-9, C3d, and C4d deposition.

Results: A few vessels demonstrated incipient platelet thrombi loosely adherent to the intimal vascular lining [fig.1a]. Patient with classic features of atypical HUS showed extensive endoluminal vascular deposits of C5b-9 [fig.1b], C3d, and C4d both immunohistochemically and by immunofluroescence.



All patients with classic features of atypical HUS responded to eculizumab therapy. With the exception of 2 patients, the remaining control group did not show significant vascular deposits of C3d, C4d, and C5b-9 nor were there obvious light microscopic abnormalities.

Conclusions: The skin biopsy defines a very important role in regards to establishing a diagnosis of atypical HUS. The pattern and extent of C5b-9 deposition was clearly abnormal in patients with diagnostic clinical features of atypical HUS, manifesting an endoluminal granular localization pattern frequently with subtle features light microscopically of an underlying procoagulant state. The common mechanism of vascular injury in atypical HUS is C5b-9 and has important therapeutic implications with respect to the implementation of complement inhibiting agents such as eculizumab. Microvascular deposits of C5b-9 likely affects many organ systems including organs without obvious features of vascular compromise.

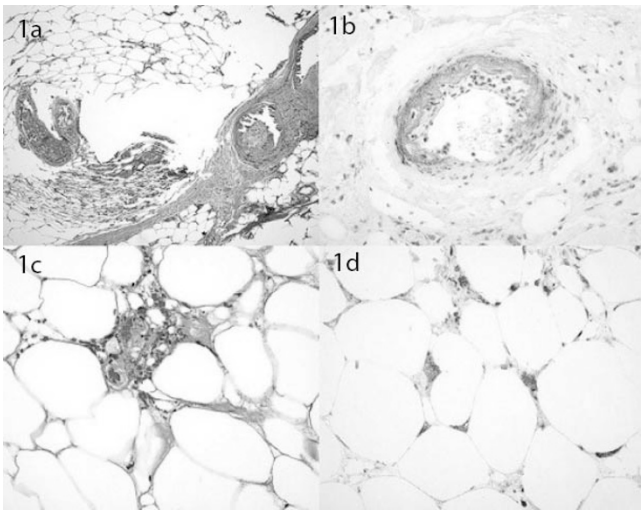
558 Osteopontin Expression in Biopsies of Calciphylaxis

S Momtahen, JW Hagen, CM Magro. Weill Medical College of Cornell University, New York, NY; University of Pittsburgh, Pittsburgh, PA.

Background: Calciphylaxis reflects a severe tissue compromise attributable to a unique microangiopathy combining features of microvascular thrombotic occlusion with endoluminal calcification. At times subcutaneous thrombosis without obvious calcification is seen. While mostly described in patients with renal failure, it is seen in other settings such as multiple myeloma, POEMS syndrome, cirrhosis and rheumatoid arthritis. Various factors contribute to this occlusive microangiopathy most notably an underlying pro-coagulant state and ectopic neo-osteogenesis of the microvasculature.

Design: 23 formalin-fixed, paraffin embedded skin biopsies with confirmed diagnosis of calciphylaxis were assessed for osteopontin expression using immunohistochemistry and Von Kossa histochemical stain.

Results: Skin biopsies of 18 females and 5 males with a mean age of 62 years of age were studied. While lower extremities were the most commonly involved areas, a truncal and genital distribution was noted in 3 cases. The majority of patients had renal failure however 1 patient had metastatic colonic adenocarcinoma and another patient had myelodysplastic syndrome without concurrent renal failure. Typical changes of a calcific endoluminal arteroipathy [fig1a] were noted as well as microvascular thrombosis without calcification [fig1c]. We found high levels of osteopontin staining in all calciphylaxis biopsies with the dominant localization of expression in the subcutaneous vasculature independent of the presence or absence of calcification. The staining was primarily endoluminal and was observed in normal appearing capillaries, thrombosed vessels [fig1d] and vessels showing calcification [fig1b]. There was a disparity between the striking extent of subcutaneous expression and lack of expression in a similar pattern in the overlying dermis.



Conclusions: An underlying procoagulant state in concert with ectopic osteopontin production work synergistically to result in calciphylaxis. Ectopic osteopontin has varied sources not limited to renal failure including its production by myeloid cells. Thrombin activates osteopontin to promote ectopic bone formation. Thus, therapeutic endeavors designed to reduce osteopontin expression may be of value in diminishing patient morbidity and mortality.

559 Cutaneous Basal Cell Carcinomas and Squamous Cell Carcinomas Frequently Harbor *TERT* Promoter Mutations

R Murali, KG Griewank, B Schilling, T Schimming, I Moller, I Moll, M Schwamborn, A Sucker, L Zimmer, D Schadendorf, U Hillen. Memorial Sloan-Kettering Cancer Center, New York, NY; University Hospital, University Duisburg-Essen, Essen, Germany.

Background: Recently, activating mutations in the *TERT* promoter were identified in up to 71% of cutaneous melanomas. Subsequently, *TERT* promoter mutations were found in a variety of other human cancers. *TERT* promoter mutations lead to increased expression of telomerase, which maintains telomere length and genomic stability, thereby allowing cancer cells to continuously divide, and to avoid senescence or apoptosis. *TERT* promoter mutations in melanoma frequently showed UV-signatures, and UV radiation is important in the pathogenesis of cutaneous basal cell carcinomas (BCCs) and squamous cell carcinomas (SCCs). In this study, we investigated *TERT* promoter mutations in BCCs and SCCs.

Design: Using conventional Sanger sequencing, we sequenced the *TERT* promoter region in DNA extracted from 32 BCCs and 34 SCCs, and analyzed associations of mutation status with clinical and pathologic features, including: age, sex, histologic subtype, tumor thickness, cystic component, ulceration status, and presence of pigmentation for BCC, as well as tumor thickness, Clark level, acantholysis, lymphovascular involvement (LVI), perineural involvement (PNI), and presence of ulceration in SCC.

Results: The tumors were obtained from 43 males and 23 females. The median age was 73.0 years for BCC and 72.6 years for SCC. *TERT* promoter mutations were identified in 18 (56%) BCCs and in 17 (50%) SCCs. The recurrent mutations were identical to those previously described in cutaneous melanoma, and showed a UV-signature (C>T or CC>TT). The commonest mutations were 250C>T and 228 C>T, together occurring in 44% of BCCs and 30% of SCCs. CC>TT mutations, considered virtually pathognomonic of UV-induction, were found in 13% of basal cell carcinomas and 18% of squamous cell carcinomas; this underscores the role for UV exposure in these common cutaneous malignancies. Apart from a small, statistically significant (p=0.046) difference in age between patients with *TERT* promoter-mutant BCCs (median 75.5 years) and those with *TERT* promoter-wild type BCCs (median 71.0 years), there were no statistically significant associations of *TERT* mutation status with clinicopathologic parameters.

Conclusions: *TERT* promoter mutations with UV-signatures are frequent (~50%) in non-melanoma skin cancer. Increased expression of telomerase plays an important role in the pathogenesis of these tumors.

560 Amelanotic Acral Melanomas: Clinicopathological, *BRAF* Mutation, and *KIT* Aberration Analysis

J-H Nam, C-S Park, Y-D Choi. Chonnam National Univeristy Medical School, Gwangju, Republic of Korea.

Background: Amelanotic acral melanoma (AAM) is very rare and difficult to diagnose both clinically and pathologically. However, it has been the subject of few investigations. We analyzed the clinicopathologic features as well as *BRAF* mutations and *KIT* aberrations in 35 Korean AAM cases.

Design: We included 28 cases of complete-type and seven cases of incomplete-type AAMs. We analyzed immunohistochemical stains such as S-100, HMB-45, or MART-1. In addition, we performed *BRAF* exons 11 and 15and *KIT* exons 9, 11, 13, and 17 mutations to detect any mutations in AAM.

Results: Twenty-six AAMs (45.7%) were located in subungual areas, 21 of which (82.9%) showed ulceration. Nodular melanoma was the most common histopathologic subtype (63.6%). The most frequent cell type affected was epithelioid or spindled. HMB-45 staining was strongly positive in 66.7% of AAMs; four (12.1%) were negative for HMB-45, three of which were complete type AAMs. *BRAF* mutations were detected in

two AAM cases, and *KIT* aberrations were found in 11 cases (33.3%). *KIT* mutations were found in four cases (11.4%), all of which were complete-type AAMs. There was a weak correlation of *KIT* aberrations and c-kit staining. Twenty patients were TNM stage I or II, and mean survival was 30.14 ± 4.54 months.
Conclusions: AAMs are difficult to diagnose both clinically and histopathologically, HMB-45 staining was sometimes negative, *BRAF* mutations were rare, and *KIT* aberrations were detected.

561 Sarcoma-Like Tumor of Head and Neck Skin

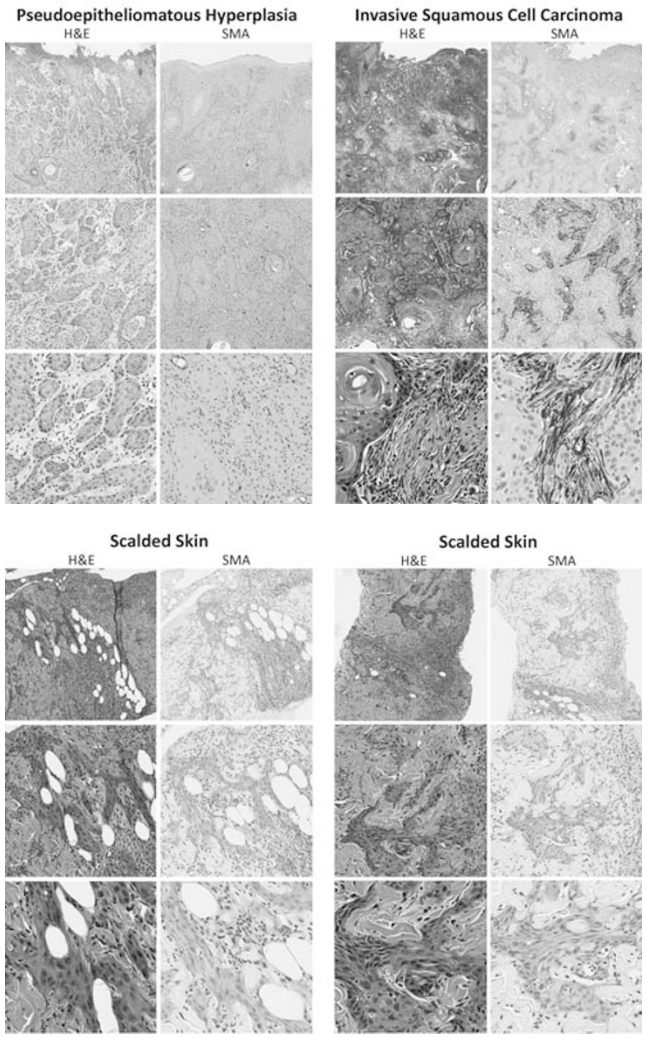
D Nonaka, PW Bishop. The Christie Hospital and Institute of Cancer Sciences, The University of Manchester School of Medicine, Manchester, United Kingdom; Wythenshawe Hospital, Manchester, United Kingdom.
Background: A group of tumor referred to as atypical fibroxanthoma (AFX) and pleomorphic dermal sarcoma (PDS) predominantly occur in sun-damaged skin of the elderly, particularly head and neck region. Although this group of tumor is often regarded as mesenchymal, the matter of histogenesis has not been entirely settled. Evans H and Smith JL reported in 1980 that the prognosis was not significantly different whether there was a definite squamous cell carcinoma (SCC) component or not, supporting a view of carcinoma in nature (sarcomatoid carcinoma, SC).
Design: One hundred sarcoma-like tumors (SLTs) of head and neck skin of the elderly, treated by wide excision, were studied. Pathology reports and H&E sections were reviewed to document size, extent of invasion, vascular and perineural invasions, margin status, ulceration, necrosis, presence of actinic keratosis (AK) in adjacent/overlying skin. Immunostains examined were; pan-CKs (AE1/AE3, MNF116), HMW-CKs (34bE12, CK5/6, CK14), p63, and melanocytic (S100, Melan A, HMB45, MITF), vascular (CD31, CD34), and muscle markers (SMA, desmin, h-caldesmon) to exclude melanoma and definite sarcoma entities. The tumors were divided to AFX/PDS (G1) and SC group, which was subdivided to SLT with only p63+ (G2a), SLT with CKs+ regardless of p63 status (G2b), and SLT with minor SCC component (G3). Clinicopathologic findings were investigated in relation to outcomes.
Results: Age at diagnosis ranged 51-96 years (median, 79), with M:F=11.5:1. There were 61 cases in G1 (20AFX, 41PDS), 30 in G2 (19 in G2a, 11 in G2b), and 9 in G3. There was no statistically significant difference in survival among all three groups. CKs and p63 expression, size, extent of invasion, VI, PNI, and ulcer did not affect outcome while margin status and necrosis did. 38 patients had multiple non-melanomatous skin cancers. AK was observed in overlying/adjacent epidermis in 51 cases. 8 patients had prior radiotherapy in head skin cancers; one SCC was transformed to sarcoma-like morphology after radiotherapy; one patient developed two separate tumors (G1 and G3) after radiotherapy. Six patients died of tumor (one G1, one G2a, two G2b, and two G3); five cases had positive margin, and one had narrow margin.
Conclusions: Our results suggest biological similarities between AFX/PDS and SC. Difference between AFX and PDS is extent of invasiveness (stage) rather than different histogenesis. AFX/PDS probably represents SC with loss of epithelial differentiation (dedifferentiation).

562 Diagnostic Difficulty of Scalded Skin and an Ancillary Role for SMA

KM Paral, P Pytel. University of Chicago, Chicago, IL.
Background: An 8-year-old male underwent skin grafting five days after third-degree burns. Light microscopy of the debrided tissue demonstrated: 1) multiple, irregular, infiltrative-appearing islands of squamous epithelium involving the dermis and subcutaneous fat; 2) cytologic atypia characterized by nuclear enlargement, hyperchromasia, irregular nuclear countours; 3) numerous mitoses. These findings were worrisome for invasive squamous cell carcinoma (SCC), but the patient's history contradicted this possibility. At present, no immunohistochemical adjunct can distinguish benign from malignant squamous epithelium, but preliminary data from our studies comparing the stroma of invasive SCC to that of pseudoepitheliomatous hyperplasia (PEH) suggest that smooth muscle actin (SMA) may provide diagnostic utility in this unusual case.
Design: Intradepartmental review was sought, and immunostaining for SMA was performed. Staining of stromal cells was compared with 15 cases of PEH, 13 cases of SCC, and 12 cases of tumor-free tissue. Staining patterns were scored as follows (excluding vessels): -= no positive cells, + = focal (<50% positive cells), and ++ = strong (>50% positive cells).
Results: The consensus opinion favored a benign process due to the clinical history. Ninety-two percent of SCC demonstrated stromal SMA positivity, whereas only 1 case of PEH (pyogenic granuloma) demonstrated SMA positivity. Diffuse stromal SMA positivity was 100% specific for SCC, and diffuse negativity was 96% specific for uninvolved stroma. The present case, being diffusely negative for SMA, stained similarly to the PEH cases, supporting the diagnosis of a benign process.

Table 1. Stromal patterns of α-SMA+ myofibroblastic cells

	-	+	++
Tumor-free	12	0	0
PEH	14	1	0
SCC	1	6	6



Conclusions: The present case underscores the inherent necessity of clinicopathologic correlation, but in cases in which clinical history is unclear or not provided, SMA offers diagnostic utility.

563 Primary Acral Lentiginous Melanoma in Patients with Non-Caucasian Ethnicities from East Asia and Latin America Demonstrates Frequent Heterogenous Immunodetection of BRAFV600E and Preserved PTEN Protein Expression

P Pattanaprichakul, S Casavilca, M Tetzlaff, L Cano, P Sitthinamsuwan, C Barrionuevo, S Kumnerdnakta, N Ramani, V Prieto, J Curry, C Torres-Cabala. Siriraj Hospital, Bangkok, Thailand; Instituto Nacional de Enfermedades Neoplasicas, Lima, Peru; UT MD Anderson Cancer Center, Houston, TX.
Background: Cutaneous melanomas (CM) typically harbor distinct activating mutations involving *BRAF*, *NRAS*, and *KIT* oncogenes in addition to activation of PI3K-Akt pathway with loss of *PTEN*. Acral lentiginous melanoma (ALM) is an aggressive type of CM enriched in non-Caucasian (NC) ethnic populations in East Asia (EA) and Latin America (LA). The presence of *BRAFV600E* in ALM from patients of Caucasian ethnicities is rare. Studies have shown that homogeneous immunodetection for BRAFV600E highly correlates with *BRAFV600E* mutation status. However, a minor subset of tumors shows heterogenous BRAFV600E staining, yet still harbors *BRAFV600E* mutation by sequencing. Our knowledge of the genetic alterations in ALM in patients with NC ethnicities is limited. We examined BRAFV600E and PTEN protein expression in ALM in patients from Thai and Peruvian ethnicities.
Design: We selected ALM from patients with Thai (N=37) and Peruvian (N=44) ethnicities and performed immunohistochemical studies (IHC) with anti-BRAFV600E and PTEN. The pattern and frequency of immunolabeling were scored for BRAFV600E as follows: 0 (no staining); 1 (<10% positive cells (PC); 2 (10-50% PC); 3 (50-90% PC); 4 (>90% PC) and for PTEN: 0 (<10% PC); 1 (>= 10% PC). Comparison of the percent of PC between ALM from Peruvian and Thai patients was made.
Results: Immunodetection of BRAFV600E and PTEN in ALM are presented in Table 1. In summary, greater than 37% of the ALM from EA and LA demonstrate tumor heterogeneity with BRAFV600E and with nearly 20% of the cases with BRAFV600E staining in more than 50% of the tumor cells. PTEN expression was preserved in 91% of ALM.

Table 1. IHC expression of BRAFV600E and PTEN in acral lentiginous melanoma

	BRAFV600E	BRAFV600E	PTEN	PTEN
Score	Thai (%)	Peruvian (%)	Thai (%)	Peruvian (%)
0	20 (54)	31 (70)	3 (8)	4 (9)
1	4 (11)	3 (7)	33 (92)	40 (91)
2	5 (14)	3 (7)		
3	4 (11)	0		
4	4 (11)	7 (16)		
Total cases (-)	20 (54)	31 (70)	3 (8)	4 (9)
Total cases (+)	17 (46)	13 (30)	33 (92)	40 (91)

Conclusions: BRAFV600E tumor heterogeneity is present in a high proportion of ALM from patients in EA and LA, suggesting possible high BRAFV600E mutation frequency. PTEN appears preserved in overwhelmingly a majority of ALM with minor subset of PTEN loss in Thai and Peruvian patients. Further molecular studies are necessary to determine the clinical significance BRAFV600E tumor heterogeneity in ALM in patients from EA and LA.

564 Immunophenotype of Hypopigmented Mycosis Fungoides

P Persad, SK Tian, OP Sangueza, B Noya. Wake Forest Baptist Medical Center, Winston Salem, NC; Central University Venezuela, Caracas, Venezuela.

Background: Mycosis fungoides (MF) is the most common form of cutaneous T cell lymphoma. Hypopigmented MF (HMF) presents deceptively as light colored macules in healthy young (20-40 years), dark-skinned (type IV) patients. Melanocytes may be a target of T cells leading to a vitiligo-like picture. HMF patients have an excellent response to phototherapy. Thus far, immunophenotypic studies of HMF have revealed mixed results with some studies demonstrating a CD8 infiltrate while others showing CD4 helper T cells. To our knowledge this is the largest multinational study comparing MF, HMF and vitiligo immunophenotype.

Design: Thirty formalin fixed paraffin embedded specimens of HMF (22), MF (4), and vitiligo (4) were retrieved from the archives of the Central University of Venezuela and Wake Forest Baptist Medical Center. Diagnostic accuracy and specimen adequacy were verified by two dermatopathologists (OS, BN). A tissue microarray of antibodies commonly used to differentiate cutaneous lymphoma cases was constructed.

Results: All cases were positive for CD45, CD2, CD3, CD5 and CD7 confirming the T cell predominance in HMF, MF and vitiligo. The mib-1 was <25% in all samples, consistent with the indolent nature of these diseases. Interestingly, all but 2 specimens demonstrated aberrant co-expression of CD4 and CD8 (1 vitiligo and 1 HMF). All cases were BF-1 positive and TCR gamma negative. B cell markers CD19, CD20, and CD79a were negative in all but one case of MF that revealed focal positivity. CD30, granzyme B, S100 and pan-mel were negative. CD117 was positive in all but one case (HMF). CD1a was also positive.

Tissue Microarray

	HMF	MF	Vitiligo
CD45/CD2/CD3/CD5/CD7	+	+	+
CD4/CD8	+	+	+
CD30/Granzyme B	-	-	-
BF-1	+	+	+
TCR gamma	-	-	-
CD19*/CD20/79a	-	-	-
pan-mel/S100	-	-	-
CD117**/CD1a	+	+	+
Mib-1	<25%	<25%	<25%

Conclusions: The immunophenotype of MF, HMF, and vitiligo are similar. Alpha/beta T cells that aberrantly co-express CD4 and CD8 were identified in all samples. Since cytotoxic T cells expressing granzyme B or large T cells expressing CD30 were not seen, a CD4 helper immunotype is favored. Melanocytic markers were negative, CD117 and CD1a were largely positive. The significance of this finding is unknown but immune targeting of melanocytes may play a role. Though the results did not allow distinction based on immune markers, the CD4 helper with alpha/ beta TCR phenotype was confirmed despite most cases demonstrating aberrant CD4/CD8 co-expression. Clinical presentation remains vital to proper diagnosis.

565 Immunologic Overlap of Th17 and Th22 Cells in Acute Psoriasis and Acute Atopic Dermatitis

A Primiani, M Murali1, D Kroshinsky, LM Duncan, RM Nazarian. Massachusetts General Hospital, Boston, MA.

Background: Psoriasis and atopic dermatitis (AD) are distinct inflammatory skin diseases that severely affect quality of life. Acute erythrodermic exacerbations of both diseases, characterized by diffuse cutaneous erythema and scaling, are associated with significant morbidity and mortality. Although psoriasis and AD are traditionally thought to be T-helper cell 1 (Th1) and 2 (Th2) mediated, respectively, and show distinct histopathologic differences during chronic disease, these diseases are clinically and histologically indistinguishable during acute exacerbations. Recently described subtypes of T-helper cells, Th17 and Th22, have been shown to be critically involved in both of these immune-mediated disorders. We investigated the T-helper cell phenotype of psoriasis and AD lesions during acute and chronic phases by immunohistochemistry.

Design: Immunohistochemical stains for CD3 and dual stains with CD4 and T-bet, GATA-3, STAT-3, or BNC-2 (transcription factors reported to be specific and mutually exclusive for Th1, Th2, Th17, and Th22 cells, respectively) were evaluated in biopsies from patients with chronic psoriasis (n=20) and chronic AD (n=20) and in biopsies from erythrodermic patients subsequently diagnosed with AD (n=5) or psoriasis (n=7). CD3-positive cells and dual-labeled CD4+/T-bet+, CD4+/GATA-3+, CD4+/STAT-3+, and CD4+/BNC-2+ cells were counted in five consecutive high power fields, independently by two authors.

Results: A significant difference in the average Th1/Th2 ratio among chronic psoriasis and chronic AD lesions was detected (0.28 and 0.09, respectively; p=0.005), with greater numbers of Th1 cells in psoriasis and Th2 cells in AD. However, there was no significant difference in the %Th1, %Th2, %Th17, and %Th22 of CD3+ T-cells or in the Th1/Th2 ratio within biopsies from erythrodermic patients with acute psoriasis and acute AD.

Conclusions: This study confirms the Th1- and Th2-skewed T-helper cell phenotype of chronic psoriasis and AD, respectively. However, we demonstrate a loss of this immune polarity, as determined by immunohistochemical analysis, in acute exacerbations of psoriasis and AD. Additionally, these quantitative analyses show no difference in the Th17 and Th22 immune phenotype of acute and chronic lesions of psoriasis and AD. Overall, these findings suggest that Th17 and Th22 are involved in the pathophysiologic mechanisms of both acute psoriasis and AD and have implications for guiding therapy.

566 Discordancy in BRAF Mutations amongst Primary and Metastatic Melanoma Lesions: Clinical and Biological Implications

JD Richey, J Bradish, KM Post, K Meehan, JD Sen, AJ Malek, TM Katona, S Warren, LA Fecher, L Cheng. Indiana University School of Medicine, Indianapolis, IN.

Background: Systemic targeted molecular therapy, in the form of a selective BRAF inhibitor, is a standard treatment for patients with $BRAF^{V600E}$ positive melanoma with unresectable stage III and IV disease. Patients with BRAF negative primary tumors with BRAF positive metastatic disease may still benefit from BRAF inhibitor therapy. It is uncertain whether metastatic melanoma, and all metastatic lesions, carry the same BRAF mutation found in the primary tumor.

Design: Primary and matched metastatic lesions in 23 melanoma patients were tested for the BRAF V600E/Ec, V600K, V600D, and V600R mutation using a BRAF RGQ PCR kit (Qiagen).

Results: Three patients (13%) had discrepancies between their primary and metastatic melanoma BRAF status. Of these patients, 2 (9%) had positive primary melanomas with BRAF negative metastatic lesions and 1 (4%) patient had BRAF negative primary melanoma with a BRAF positive metastatic lesion.

Conclusions: Discordancy of BRAF mutation status is not an infrequent finding between primary and metastatic melanoma. It is recommended to test the most recent tumor specimen for BRAF mutation status to determine treatment options, and it may be reasonable to test more that one specimen. Discordant BRAF status may play a role in the varying patterns of response and secondary resistance seen with selective BRAF inhibitor therapies. Future studies are needed to evaluate the optimal therapeutic strategy for patients with disparate BRAF mutational status.

567 The Clinical Impact of a Gene Expression Signature That Differentiates Benign Nevi from Malignant Melanoma

C Rock, K Moyes, MB Warf, S Leachman, LE Clarke. Myriad Genetic Laboratories, Inc, Salt Lake City, UT; Oregon Health & Science University, Portland, OR.

Background: Accurate classification of ambiguous melanocytic tumors continues to prove challenging when standard histopathologic criteria are used for diagnosis. In an effort to address this problem, pathologists have sought more sensitive and objective diagnostic methods for distinguishing malignant tumors from benign nevi. An expression score corresponding to a 14 gene signature, which includes 13 genes with known immune function and one that controls cell differentiation, has been developed to accurately and objectively differentiate benign and malignant melanocytic tumors. The primary objective of this study was to assess the impact of this signature on diagnostic and clinical decision-making among pathologists evaluating melanocytic tumors with ambiguous characteristics.

Design: Fifty melanocytic neoplasms with ambiguous histopathologic features were selected by a dermatopathologist from an archived cohort of 464 lesions. Digitalized slides of the ambiguous neoplasms were then evaluated by seven expert dermatopathologists. For each specimen, the pathologist recorded predicted biologic behavior, diagnosis, level of confidence in predicted behavior, any further evaluation to be performed, and recommendations for management. The pathologists then repeated the process with the gene expression score and corresponding malignant potential of the lesion available to them. Changes in pathologist assessment were measured.

Results: Clinical assessment of biologic behavior among all pathologists was concordant in only 30% of cases. Additionally, ten of the fifty cases (20%) were either classified by the majority of pathologists as indeterminate or there was no majority consensus on diagnosis. When gene expression scores were provided, predicted biologic behavior was revised in 30% of cases. Revisions in predicted biologic behavior subsequently resulted in modified management recommendations in 33.1% of cases, with a trend toward recommendations for less invasive interventions.

Conclusions: A novel molecular assay capable of differentiating malignant tumors from benign nevi impacts diagnosis and management decisions made by dermatopathologists. Integration of this assay into current pathology practice has the potential to enhance patient care through more definitive diagnoses of melanocytic lesions and personalized, cost-effective medical management based on a patient's malignant risk.

568 Clinicopathologic Findings in IgE (Anti-FcεR1alpha) Autoimmune Related Chronic Urticaria

T Rojanapremsuk, S Kasprowicz, E Schafer, R Story, MS Clarke, TB Walls, VS Snyder, AB Thomas, TL Cibull. NorthShore University HealthSystem, Evanston, IL.

Background: Urticaria is a common condition affecting approximately 15% of the population characterized by pruritic, edematous, erythematous papules or wheals. Most cases of urticaria occur in children and young adults and are acute. Chronic urticaria (CU) (> 6 wks) is less common and is usually seen in middle-aged adults with female predominance. 50-75% cases of CU are idiopathic; however, some causes for CU have been identified. One cause of CU is autoreactivity which is of autoimmune origin and is diagnosed by the detection of autoantibodies directed against the IgE receptor alpha subunit (anti-FcεR1alpha). Patients with autoimmune CU tend to be resistant to

antihistamine drugs and may require immunosuppression. The objective of our study was to compare clinicopathologic features of CU patients testing positive for anti-IgE receptor antibody (Ab) to those testing negative.

Design: 438 patients (2011-2013) had anti-IgE receptor Ab tested and 37 patients had skin biopsy for evaluation of CU. Demographics and histologic characteristics (predomination of eosinophils or neutrophils; presence or absence of vasculitis) were reviewed and compared between negative and positive anti-IgE receptor Ab groups.

Results: 1. Out of 438 patients, 146 (33%) cases had positive anti-IgE receptor Ab and 292 (67%) cases had negative anti-IgE receptor Ab.

2. Skin biopsy and patient demographic results are listed in tables 1 and 2.

Patient demographics

Total Number of Patients	Mean Age	Female	Male
37	45 (range 13-82)	27 (69%)	12 (31%)
Positive anti-IgE receptor Ab = 18	43 (range 14-71)	12 (75%)	6 (25%)
Negative anti-IgE receptor Ab = 19	47 (range 13-82)	14 (74%)	5 (26%)

Summary data

Anti-IgE receptor Ab	Eosinophil predominant	Neutrophil predominant	Vasculitis
Positive	13	5	None
Negative	10	9	None

Conclusions: 1. 33% of our study population had IgE related autoimmune urticaria. Similar to CU as a whole, autoimmune CU was more common in middle-aged females than in males.

2. There were no significant histologic differences between anti-IgE receptor Ab positive and negative cases.

3. There were three cases demonstrating > 50 neutrophils per HPF in the anti-IgE receptor Ab positive group. There was no significant difference between anti-IgE receptor Ab levels in these three patients compared with the group as a whole.

4. In patients with CU, histologic findings do not differentiate between autoimmune related CU and idiopathic CU. Therefore, serum testing for anti-IgE receptor Ab is required to identify this subgroup of CU patients.

569 BRAF V600E Immunohistochemistry in Cutaneous Langerhans Cell Histiocytosis

RC Romano, W Shon, KL Fritchie. Mayo Clinic, Rochester, MN.

Background: Langerhans cell histiocytosis (LCH) is a rare proliferative disorder with a wide spectrum of clinical manifestations. 40% of patients with LCH develop cutaneous involvement. Oncogenic BRAF V600E mutations have been implicated in the pathogenesis of LCH, supporting the theory that LCH is a neoplastic process. BRAF mutations have been evaluated in LCH using molecular techniques, and BRAF V600E immunohistochemistry has been employed in evaluation of other diseases. We explore the presence of BRAF V600E immunoreactivity in a series of cutaneous LCH with emphasis on clinicopathologic correlation.

Design: Twenty dermatologic biopsy specimens from 14 patients (mean age 31.3 yrs, range 1-78 yrs) diagnosed as LCH were obtained from the Mayo Clinic archives. Controls included 4 cases of other dermatoses. Formalin-fixed, paraffin-embedded sections were immunostained with mouse monoclonal BRAF V600E antibody (clone VE1, 1:100, Spring Bioscience, Pleasanton, CA) using heat-induced epitope retrieval and visualized using Ventana (Tucson, AZ) OptiView TM amplification and detection kits. Clinical information obtained from records was correlated with immunostaining profiles.

Results: Overall, BRAF V600E staining was identified in 15 of 20 (75%) specimens of cutaneous LCH across all age groups. In patients with multiple biopsies, the biopsy results were concordant. No control cases were positive (0/4). In the pediatric group, positive BRAF V600E staining was identified in 4 of 5 patients (80%). Two male pediatric patients had lytic skull lesions, and the cutaneous biopsies from both of these patients were positive for BRAF V600E. The pediatric patient with negative staining presented with diabetes insipidus. In the adult group, BRAF V600E staining was positive in 5 of 9 patients (56%), and no correlation with sex, location, distribution or size of lesion was appreciated.

Conclusions: Although a slightly higher rate of positivity was noted in the pediatric population (80% vs. 56%), no signification correlation with site or distribution of disease was identified in either pediatric or adult groups. However, the presence of BRAF V600E immunostaining in 15 of 20 cases of cutaneous LCH cases and absence of staining in control cases suggests that it is a relatively sensitive (75%) and specific (100%) marker of the disease ($p =< 0.05$). Furthermore, BRAF V600E immunostaining may be a cost-effective method to determine which patients could benefit from targeted therapy.

570 Malignant Melanoma of the Nail Unit: A Fluorescence In Situ Hybridization (FISH) Analysis of 7 Cases

R Romano, W Shon, W Sukov. Mayo Clinic, Rochester, MN.

Background: Malignant melanoma of the nail apparatus is exceedingly rare, with an estimated prevalence of 0.7-3.5% of all melanomas. Diagnosis, traditionally rendered through histopathologic examination of tissue biopsies, must be accurate and timely to prevent unfavorable outcomes. Increasingly, genetic studies have been employed to aid in distinguishing between malignant melanoma and benign melanocytic nevi. The most common abnormalities associated with melanoma are copy number increases of 6p25 (RREB1), 11q13 (CCND1), and 8q24.1 (MYC), as well as loss of 6q23 (MYB) and 9p21 (CDKN2A). We aim to evaluate these genetic aberrations as they relate to nail unit melanomas using a fluorescent in situ hybridization (FISH) assay.

Design: Archived nail unit malignant melanoma surgical specimens obtained at Mayo Clinic from 2000-2013 were retrieved. Appropriate controls were employed. Formalin-fixed, paraffin-embedded sections from 10 cases (7 experimental, 3 negative controls) were analyzed by FISH using probes targeting the genes at 6p25 (RREB1), 11q13 (CCND1), 8q24.1 (MYC), 6q23 (MYB), 9p21 (CDKN2A), and the centromeres of chromosomes 8 (D8Z2) and 6 (D6Z1). The results were correlated with clinical and demographic information obtained from our records.

Results: Demographic results for the experimental group were as follows: mean patient age, 57.8 (range 23-92); 3 males, 4 females. 5/7 (71.4%) cases involved the upper extremity digits. RREB1 and CCND1 gains were seen in all cases. Remaining abnormalities were seen in decreasing frequency as follows: MYB loss (6/7, 87%), MYC gain (5/7, 71%), RREB1 gain relative to centromere 6 (4/7, 57%), homozygous loss of CDKN2A (1/7, 14%). 2/7 (28.6%) patients had lymph node metastasis and died of widely metastatic disease. These two patients harbored the most genetic aberrations: gains of RREB1, CCND1, and MYC, and MYB loss.

Conclusions: Melanoma of the nail unit appears to occur more frequently in the upper extremities. There is no significant gender predilection. Homozygous loss of CDKN2A (9p21) is uncommon in nail unit melanoma, in contrast to non-acral melanomas. RREB1 and CCND1 gains are common as in most melanomas, and an increased number of genetic aberrations are associated with a poorer prognosis. FISH is a useful adjunct in the evaluation of diagnostically challenging melanoma of the nail unit, and may highlight an aberrant genetic profile that is slightly different from that of non-acral melanoma.

571 Mixed Tumor of the Skin: Its Clinicopathological and Immunohistochemical Characteristics

M Shimizu, L Jin, H Yamaguchi, K Nagata, T Hasebe, A Sasaki, M Yasuda. Saitama Medical University, International Medical Center, Hidaka, Saitama, Japan; Saitama Medical University, Moroyama, Saitama, Japan.

Background: Mixed tumor of the skin is also known as chondroid syringoma. Although several histological variations have been reported, ossification has rarely been observed. In addition, immunohistological staining of c-kit has not been reported in mixed tumor of the skin. Here we reviewed its clinicopathological and immunohistochemical characteristics.

Design: Thirty-three cases of mixed tumor of the skin were reviewed regarding the age and sex of the patient, and the tumor's site, size, histology, and immunostaining of c-kit. Histologically, two components, namely epithelial and stromal elements, were evaluated in detail.

Results: The average age of the patients studied was 58 years (range of 21 to 90 years), and the male-to-female ratio was almost 1:1. Microscopically, most cases were located in the dermis and/or subcutis as a well-demarcated lobular lesion. No connection to the epidermis was observed. Tumors were composed of an epithelial component and myxoid stroma. The former showed various-sized glandular structures with two cell populations. Myoepithelial cells, especially hyaline/plasmacytoid cells, were observed as myoepithelial cells. Regarding the stroma, a chondroid portion was also seen. Three cases (9%) showed extensive ossification, and seven cases (21%) revealed calcification, which was of a higher incidence compared to that of reported cases. A keratocyst-like structure was observed in 19 cases (58%), and mature fat cells were seen in 25 cases (76%). Immunohistochemically, both epithelial cells and hyaline/plasmacytoid cells were positive for c-kit.

Conclusions: Although the sex ratio is reported to be 3:1, no sex predilection was observed in our cases. Extensive ossification was noted in 9% of the cases, which was a rather high incidence. It is very interesting that c-kit was positive for both epithelial cells and stromal cells (especially hyaline/plasmacytoid cells). Although the precise etiology still remains unknown, the presence of c-kit-positive cells may be involved in its pathogenesis of mixed tumor of the skin.

572 Primary Dermal Melanoma: A Clinical, Histopathologic, Fluorescence In Situ Hybridization and mRNA Expression Profiling Study of 50 Cases

M Sidiropoulos, R Obregon, LM Sholl, J Guitart, P Gerami. Northwestern University, Feinberg School of Medicine, Chicago, IL.

Background: Primary dermal melanoma (PDM) is a subtype of melanoma confined to the dermis and/or subcutaneous fat that may be difficult or impossible in some cases to distinguish from cutaneous melanoma metastases (MM). PDM is associated with an unusually high survival rate, making differentiating PDM from MM critical. In recent times, molecular diagnostics including fluorescence in situ hybridization (FISH) and mRNA expression profiling have emerged as promising diagnostic aids.

Design: In this retrospective study, we examined the clinical, histopathologic, and molecular findings in 50 cases of PDM and compared them to 20 cases of MM. A 6-probe FISH assay targeting 6p25 (RREB1), 6q23 (MYB), Cep6 (centromere 6), 11q13 (CCND1), Cep9 (centromere 9) and 9p21 (CDKN2A) and Castle mRNA expression profiling was utilized. Clinical history and findings of PDM from workup were negative for evidence of melanoma elsewhere.

Results: The PDM cohort included 23 men and 27 women, ranging in age from 8 to 83 years (median 51 y). The most common anatomic site of involvement was the trunk (37%) followed by the head and neck (33%), lower extremities (18%) and upper extremities (12%). Histologically, there was no evidence of an overlying in situ component or regression. The mean Breslow depth was 4.0 mm and Clark's level IV. The mean mitotic rate was 4.6 mitoses per mm3 and focal ulceration was present in only 3 cases (6%). An associated nevus was identified in 21 cases (42%). Angiotropism (5/50; 10%), angioinvasion (2/50; 4%) and perineural invasion (3/50; 6%) were uncommonly identified. Spitz morphology was noted in 15 cases (30%). Criteria for FISH were met in 20 of 28 cases analyzed (71%). Increased number gains in RREB1 (14/20), CCND1 (9/20) and MYB (3/20) and homozygous 9p21 deletion (6/20) were identified. MRNA expression profiling using the Castle prognostic test for melanoma shows that while nearly all MM are class II, PDM may be class I. Follow-up to date revealed metastatic disease in less than 20% of PDM cases.

Conclusions: We believe our study offers some useful clinical, histologic and molecular clues to help differentiate PDM from MM.

573 Clinical Next-Generation Sequencing Reveals Divergent Mutational Patterns in *BRAF* V600, *BRAF* Non-V600, and *NRAS* Mutations in Advanced Melanoma

AE Siroy, VG Prieto, JE Gershenwald, SE Woodman, J Roszik, KD Aldape, R Luthra, KP Patel, MJ Routbort, MA Davies, AJ Lazar. University of Texas MD Anderson Cancer Center, Houston, TX.

Background: The majority of melanomas harbor somatic mutations in the *BRAF* or *NRAS* genes in the MAPK pathway. The majority of *BRAF* mutations affect the V600 locus, and most previous studies have focused on their molecular and clinical associations. However, relatively little is known about the nature and associations of *BRAF* mutations at other loci in the gene. As an initial approach to understand the differences between *BRAF V600, BRAF* non-V600, and *NRAS* mutations we reviewed the molecular testing results of advanced melanoma patients who underwent molecular testing at our cancer center since the institution of clinical next-generation sequencing (NGS) of commonly mutated regions in 46 genes.

Design: Clinical NGS of commonly mutated regions of 46 genes was performed on advanced melanoma samples in a CLIA-lab and reported in the patient's medical record. Under an IRB-approved protocol, the clinical NGS data and clinical features of 699 consecutive melanoma samples (699 pts) performed at our institution were tabulated for analysis.

Results: 80% of pts had at least one gene mutation present, with 31% having two or more mutations. *BRAF* V600 (36%), *NRAS* (21%), *TP53* (15%), and *BRAF* non-V600 (5%) were the most common mutations identified. Concomitant mutations were present in a substantial subset of patients with *BRAF* non-V600 (55%), *BRAF* V600 (32%), and *NRAS* (29%) mutations. *TP53* mutation was the most prevalent concomitant mutation in *NRAS* (21%) and *BRAF* V600 (11.5%) tumors, and the second most prevalent in BRAF non-V600 mutant (13%) tumors. *BRAF* non-V600 tumors frequently had concomitant *NRAS* (18%; most prevalent) or *KRAS* (11%) mutations; in contrast, only 1.6% of melanomas with *BRAF* V600 mutations had a concurrent *NRAS* mutation, and none had *KRAS* mutations.

Conclusions: *BRAF* V600, *NRAS*, *TP53*, and *BRAF* non-V600 were the highest frequency mutations observed in a large cohort of melanomas tested with the NGS 46 gene panel. *TP53* was the most prevalent concomitant mutation in melanomas with *BRAF* V600 and *NRAS* tumors. In contrast, concomitant mutations in *NRAS* and *KRAS* (combined 29%) were very frequent in melanomas with *BRAF* non-V600 mutations. The results demonstrate the significant molecular heterogeneity of melanoma, and provide information for future interrogation of clinical associations with the observed molecular events.

574 Evaluation of T Regulatory Cells in Regressing Melanocytic Neoplasms

JJ Speiser, L Blumenthal, S Kliethermes, KM Mudaliar, KA Hutchens. Loyola University Medical Center, Maywood, IL.

Background: Regression is a complex process by which the body targets and destroys neoplastic cells, likely through an immune mechanism. T regulatory cells (T_{reg}) have a well-documented role in immune modulation and have been shown to be increased in malignant melanoma and decreased in autoimmune conditions. A change in the quantity of T_{reg} may also play a role in spontaneously regressing melanocytic lesions (SRML). Our study compares the quantity of T_{reg} in partially spontaneously regressed malignant melanoma (MM), halo nevi (HN) and lichen planus like keratosis (LPLK).

Design: We evaluated 7 MM, 9 HN and 13 LPLK formalin-fixed, paraffin-embedded specimens via immunohistochemical double staining. The cases were of similar histologically identifiable stages to account for inter-specimen inflammatory variability. Two independent observers counted the total number of $CD4^+FoxP3^+$ and $CD25^+FoxP3^+$ cells from the three most dense 50X areas of inflammation. All of the cases were averaged and compared for statistical significance.

Results: There was a statistically significant reduction in the mean quantity of $CD4^+FoxP3^+$ and $CD25^+FoxP3^+$ T_{reg} in MM and HN when compared to LPLK and no significant difference between MM and HN.

Conclusions: T_{reg} are reduced in SRML, which parallels studies on vitiligo that demonstrate that reduced T_{reg} cause decreased suppression of T cells that target and destroy melanocytes. T_{reg} are reduced when compared to LPLK, suggesting that regression may have different immunologic mechanisms depending on the lineage of the neoplasm. These findings are important for understanding the inflammatory milieu in regression with future goals for implementation in immune therapy.

575 Array Comparative Genomic Hybridization Analysis of Congenital Melanoma: A Case Series of 3 Patients

A Su, J Kerstetter, L Low, X Li, RL Barnhill. UCLA, Los Angeles, CA; Loma Linda University, Loma Linda, CA.

Background: Congenital melanoma is extraordinarily rare. According to a literature review, 27 cases have been reported. Three types have been described: transplacental metastases from the mother, de novo congenital melanoma, and melanoma occurring in association with a congenital melanocytic nevus. Comparative genomic hybridization (CGH) is a powerful adjunctive tool in the diagnosis of difficult melanocytic lesions. We describe high-resolution array CGH analysis of 3 cases of congenital melanoma (de novo), including the first three reports of CGH analysis of de novo congenital melanoma.

Design: Routine H&E sections and immunohistochemistry from 3 suspected cases of congenital melanoma were reviewed. Patient 1 was male, and patients 2 and 3 were female. All had a scalp lesion present at birth which grew quickly. The excision specimens from cases 1 and 2 showed malignant-appearing melanocytic neoplasms without evidence of a congenital nevus. Case 3 showed a severely atypical pigmented epithelioid cell melanocytic proliferation with background features suggesting blue nevus. Patients 1 and 2 developed metastases. Patient 1 was treated with chemotherapy but expired at age 5 months. Patients 2 and 3 are currently alive at ages 3 years and seven months, respectively. Array CGH for all cases was performed with the Agilent SurePrint G3 ISCA CGH+SNP Microarray Kit, 4x180K utilizing DNA extracted from formalin-fixed and paraffin-embedded tissue. Gender-matched genomic DNA from Promega was used as controls. The data was analyzed with Agilent Cytogenomics Edition software.

Results: The first case displayed losses of 3p26.3-p21.31, 5p15.33-q23.1, 11q15.5-q13.2, 14(complete deletion), and 15q11.1-q22.31. The second case displayed gains of 1q21.1-q44, 2p25.3-p11.1, 2q11.1-q37.3, 6p25.3-p11.1, 7p22.3-p11.2, 7q11.1-q36.3, 8p23.3-p11.1, 8q11.1-q24.3, 9p24.3-p11.2, 9q12-q34.3, 11q13.2-q13.4, 13q11-q34, 18p11.32-p11.21, 19p13.3-p11, 19q11-q13.43, 20p13-p11.1, and 20q11.21-q13.33. The third case displayed losses of 1p36.33-p35.3, 1q32.1-q44, and 17q11.1-q24.2. In the second case, gains of 1q, 6p, 7, 8q, and 20q are known to be recurrent aberrations in melanoma.

Conclusions: Multiple chromosomal aberrations in all cases corroborated the diagnosis of melanoma and highlighted the utility of array CGH. The third case suggested the entity "animal type" melanoma. Given the lack of overlap among the results of the cases, it appears that different genetic pathways may be involved in congenital melanoma.

576 A Newly Developed Mouse Monoclonal SOX10 Is a Highly Sensitive Marker for Malignant Melanoma, Including Spindle Cell and Desmoplastic Melanoma

D Tacha, R Bremer, C Yu, L Hoang, S Ra, B Robbins. Biocare Medical, Concord, CA; San Diego Pathologists Medical Group, San Diego, CA.

Background: SOX10 is a nuclear transcription factor that participates in neural crest development and the differentiation of cells of melanocytic and schwannian lineage. SOX10 is expressed in malignant tumors such as melanoma, malignant peripheral nerve sheath tumors, and a subset of breast carcinomas. Importantly, SOX10 has been shown to be a sensitive and specific marker for spindle cell and desmoplastic melanomas. However, publications for SOX10 are limited to a goat polyclonal antibody, which may hinder its acceptance in a clinical setting, as polyclonal antibodies have shown lot-to-lot variation. In this study, a mouse monoclonal SOX10 antibody has been developed for immunohistochemistry (IHC) and was evaluated for sensitivity and specificity.

Design: Tissue microarrays of malignant melanoma, spindle cell and desmoplastic melanoma, schwannoma and nevus were evaluated by IHC using mouse monoclonal SOX10. Normal and neoplastic tissues were evaluated for specificity.

Results: In normal tissues, SOX10 stained skin melanocytes, myoepithelial cells in breast and salivary gland, peripheral nerves, and brain. SOX10 also stained neuroendocrine cells throughout the digestive tract. SOX10 stained 200/219 (91.3%) melanomas (Table 1). Notably, 23/24 (95.8%) of spindle cell and desmoplastic melanomas were positive for SOX10. In addition there was 100% staining for schwannomas and nevi. In other neoplasms tested (n=628), SOX10 was expressed in 18/109 (16.5%) infiltrating ductal breast cancers, and in none of the following (n=413) other carcinomas, including lung, colon, prostate, bladder, kidney, liver, esophagus, seminoma, ovary, adrenal, thyroid, pancreas and cervix. SOX10 was positive in 2/21 rhabdomyosarocomas, 1/21 of leiomyosarcomas and 29/51 (56.9%) of CNS gliomas. Carcinoid tumors in the digestive tract and in the lung were all negative, except for staining of sustentacular cells.

Table 1

Diagnosis	Cases	SOX10 (+)	% (+)
Melanoma (skin)	109	105	96.3%
Metastatic melanoma	86	72	83.7%
Spindle cell melanoma	9	9	100%
Desmoplastic melanoma	13	12	92.3%
Nevi (various types)	20	20	100%
Schwannoma (neurilemmoma)	28	28	100%
Brain	51	29	56.9%
Breast carcinoma	109	18	16.5%
Other carcinomas	413	0	0%
Rhabdomyosarcoma	21	1	4.8%
Leiomyosarcoma	21	2	9.5%.

Conclusions: This is the first report of a newly developed mouse monoclonal SOX10. In this study, SOX10 was a highly sensitive and specific marker for melanoma and its variants, including desmoplastic and spindle cell melanomas; and was also expressed in a subset of breast cancers.

577 The Utility of GATA3 in Distinguishing Breast Ductal Carcinoma from Skin Adnexal Neoplasms

MP Tilson, PB Illei, P Argani, JM Taube, A Cimino-Mathews. Johns Hopkins Hospital, Baltimore, MD.

Background: GATA3 is a transcription factor involved in the differentiation of numerous tissue types including skin, breast and urothelium. Immunohistochemistry (IHC) for GATA3 has shown good sensitivity for breast ductal carcinoma in multiple studies. In the setting of a superficial lesion, primary or metastatic breast carcinoma can be difficult to distinguish histologically from skin adnexal neoplasms. As treatment and prognoses differ greatly in these tumor types, accurate diagnosis is critical. GATA3 is expressed in benign squamous epithelium and hair follicles, but to our knowledge has never been studied in skin adnexal neoplasms. Here, we report the first systematic study of GATA3 expression in 93 cases of adnexal tumors.

Design: Departmental files were searched for cases of follicular (n=6), sebaceous (n=22), eccrine (n=13) and apocrine (n=26) adnexal neoplasms. Follicular neoplasms included trichoblastomas and trichoepitheliomas; sebaceous neoplasms included carcinomas (n=19) and adenomas (n=3); eccrine neoplasms included syringomas, benign mixed tumors and poromas; apocrine neoplasms included apocrine carcinoma (n=1), microcystic adnexal carcinoma (MAC, n=4), cylindromas, hidradenoma papilliferum,

spiradenomas, and acrospiromas/hidradenomas. Whole sections were labeled for GATA3 by IHC. Positive nuclear labeling was scored as diffuse (>50% cells) or focal (<50% cells), with any intensity considered positive.
Results: Differing GATA3 labeling patterns were seen among the adnexal tumor groups. Nearly all tumors of follicular-origin (100%) and sebaceous-origin (95%) demonstrated diffuse positive GATA3 labeling. In contrast, GATA3 labeling was variable in the eccrine and apocrine tumor groups, with most tumors showing focal or negative labeling. Seventy-seven percent of eccrine neoplasms and 81% of apocrine neoplasms displayed only focal or absent GATA3 labeling. Of the malignant apocrine neoplasms, all MAC showed diffuse GATA3 labeling, while the one apocrine carcinoma showed focal weak labeling.
Conclusions: GATA3 shows differential labeling patterns in skin adnexal neoplasms, with most follicular and sebaceous neoplasms demonstrating strong diffuse labeling and most eccrine and apocrine neoplasms demonstrating absent or focal labeling. When used in conjunction with histologic features and a panel of immunostains (e.g., p63, D2-40, mammaglobin), the presence of GATA3 labeling in an apocrine lesion may favor a breast primary, but it is not entirely specific. Additionally, the majority of cases in this study were benign adnexal neoplasms, and further studies on adnexal apocrine carcinomas are warranted.

578 P62 and Ubiquitin Expression in Melanoma: Correlation with Prognosis and Survival

RG Tipton, C Kovach, L Stuart, K Fisher, C Cohen. Emory University, Atlanta, GA.
Background: Autophagy has been shown to be a protective mechanism for tumor cells under stressful conditions and as such has been the subject of numerous therapeutic studies. P62 has proved to play an important role in this protective process by binding to ubiquitinated proteins and chaperoning them into lysosomes for degradation. Autophagy-defective human tumor cells have been show to undergo several changes, from mitochondrial and genomic damage to accumulation of p62. Studies in melanoma have demonstrated that markers of autophagy may correlate with invasiveness, resistance to chemotherapy and survival. We studied melanomas for p62 and ubiquitin expression correlating results with prognostic parameters and outcome.
Design: 62 melanomas in tissue microarrays, immunostained for p62 and ubiquitin, were evaluated as percent positive cells (0-100%), intensity (0-3+), and localization (nuclear, cytoplasmic, or both) for both antibodies. Statistical comparisons were made between expression, prognostic parameters, and survival using T-test and/or chi-square tests for continuous or categorical/ordinal variables, respectively. Comparison between Kaplan-Meier survival curves were made with a log rank test.
Results: 81% (n=50) of melanomas stained with p62, 30% (n=19) with cytoplasmic stain, and 45% (n=28) with nuclear and cytoplasmic stain; 56% (n=35) exhibited high intensity (2-3+) stain. 94% (n=58) of melanomas stained for ubiquitin with the majority exhibiting both nuclear and cytoplasmic staining (n=46, 74%). 40% of melanomas showed concordant staining of p62 and ubiquitin. High (2-3+) intensity staining with p62 correlated with high pT (Breslow p=0.0457 and Clark p=0.0575) and metastasis (p=0.0404) but not with survival (p=0.2102). Low to absent ubiquitin stain intensity (0-1+) reflected a survival disadvantage (p=0.0358).
Conclusions: P62 and ubiquitin expression were present in the majority of melanomas, suggesting that autophagy may be involved in melanoma carcinogenesis. Additionally, staining characteristics correlated with poor prognostic parameters (depth of invasion, metastases, p62), and worse survival (ubiquitin).

579 All That Sloughs: A Histologic Review of Cutaneous Desquamating Diseases

T VandenBoom, A DiLorenzo, KA Hutchens. Loyola University Medical Center, Maywood, IL.
Background: Cutaneous desquamating dermatitis is an umbrella term for a number of conditions that present with skin sloughing, which often display high rates of morbidity and mortality regardless of etiology. One of the most commonly seen is Stevens-Johnson Syndrome/Toxic Epidermal Necrolysis (SJS/TEN). Therapeutic modalities are controversial and range from supportive care only to administration of immunoglobulin or steroids. The differential diagnosis for this clinical presentation is broad and treatment varies greatly depending on the histologic findings. Sloughing diseases that clinically mimic SJS/TEN include acute generalized exanthematous pustulosis (AGEP), drug eruptions, psoriasis, bullous pemphigoid, pemphigus variants, and dermatitis. At Loyola we treat SJS/TEN with supportive care, making the correct histologic diagnosis imperative. The rarity of these conditions causes a subsequent lack in experience of clinicians and pathologists that diagnose them. As a referral center for desquamating conditions, Loyola has a unique experience in the diagnosis of these patients. We performed a retrospective analysis of these cases in order to better understand the histological breadth of cutaneous sloughing disorders.
Design: All biopsied cases of cutaneous sloughing from our burn unit with the clinical diagnosis of rule out SJS/TEN since 2010 were identified by our electronic database. A total of 95 cases were identified and their final diagnoses were confirmed and recorded. A chart review was performed to ensure that the histologic diagnosis was compatible with the clinical presentation and hospital course.
Results: A little over half (53%) of patients with cutaneous sloughing had histologic findings consistent with SJS/TEN. The other 47% of cases were not SJS/TEN. There were 24 patients who had drug eruptions: 16 pustular drug eruptions, 7 simple drug eruptions, and 1 bullous drug eruption. Other diagnoses included 4 patients with bullous pemphigoid, 5 patients with non-specific dermatitis, 4 patients with staphylococcal scalded skin syndrome, 2 patients with pemphigus, and 6 patients with other diagnoses including varicella infection, Sweet's syndrome, calciphylaxis, folliculitis, and leukocytoclastic vasculitis.
Conclusions: A variety of diseases mimic SJS/TEN and many of these entities are best treated with steroids. Differences in treatment regimens underscore the need for biopsy of patients with cutaneous sloughing for correct histologic classification. Pathologists and clinicians alike need to be adept at differentiating cutaneous sloughing disorders to ensure optimal patient care.

580 Specificity of Dermal Mucin in the Diagnosis of Lupus erythematosus: Comparison with Normal Skin and Other Common Dermatitides

JG Vincent, MP Chan. University of Michigan, Ann Arbor, MI.
Background: Increased dermal mucin is one of the classic features of lupus erythematosus (LE), however the amount and the distribution of mucin in this setting has not been well characterized. Differentiation of LE from other dermatitides can be challenging when scant to moderate amounts of dermal mucin is present, but other classic features of LE are minimal or equivocal. To address this diagnostic dilemma, we sought to determine the specificity of dermal mucin by comparing the amount and location of mucin in LE, normal skin, and a variety of relatively common dermatitides.
Design: Skin specimens from 103 patients were classified into 5 categories: LE (discoid, subacute cutaneous, and tumid types; n=17), eczema/psoriasis (n=15), other interface dermatitides (lichen planus, graft-versus-host disease, erythema multiforme; n=24), other perivascular dermatitides (urticaria, polymorphous light eruption [PMLE]; n=19), and normal skin (with and without solar elastosis; n=28). The amount of dermal mucin was scored on H&E and alcian blue (AB) stains as absent (0), scant (1), moderate (2), or abundant (3) in the papillary dermis (PD), superficial reticular dermis (SRD), and deep reticular dermis (DRD), respectively. H&E and AB scores were combined to give a total score of 0-6. Chi-square tests and two-tailed t-tests were performed.
Results: The mean scores in the SRD and DRD are significantly higher in LE compared to other categories ($p \leq 0.0451$). There is no significant difference in the PD scores in LE versus normal skin (p=0.2057) and other perivascular dermatitides (p=0.4031). A total score of 4+ in the SRD only gives a specificity of 58% for LE. The specificity increases to 88% when a total score of 5+ is used as a cutoff. Of all dermatitides, PMLE shows the greatest overlap with LE (mean SRD scores, 3.6 vs. 4.6; p=0.0431); a combined score of 5+ is required to distinguish the two (p=0.0414). There is no significant difference in mucin deposition in normal skin with and without solar elastosis (p=0.2622).
Conclusions: Scant to moderate amounts of dermal mucin has a limited specificity for LE. An abundant amount of mucin in the reticular dermis on H&E and/or AB stains is required to effectively exclude other dermatitides. Both SRD and DRD are the preferred sites for mucin evaluation, as mucin in the PD fails to distinguish LE from normal skin and other perivascular dermatitides. The degree of mucin deposition does not appear to be related to sun damage.

581 Can SOX-10 or KBA.62 Replace S100 Protein in Immunohistochemical Evaluation of Sentinel Lymph Nodes for Metastatic Melanoma

E Vrotsos, J Alexis. Mount Sinai Medical Center, Miami Beach, FL.
Background: Microscopic evaluation of sentinel lymph nodes for metastatic melanoma relies, in part, on the use of immunohistochemistry to identify minute metastatic deposits that may be overlooked on routine microscopy. At present S100 protein is widely used in this role, in large part for its superior sensitivity, however, interpretation is hampered by the presence of benign S100 protein positive cellular elements present in every lymph node, leading to reduced specificity and consequent deficiencies in interpretation. In recent years, multiple melanocytic markers have emerged that promise superior sensitivity and specificity, including KBA.62 and SOX-10. SOX-10 shows a nuclear pattern of staining. In normal tissue it is expressed in Schwann cells, melanocytes, and myoepithelial cells of salivary, bronchial, and mammary glands. KBA.62 is also specific except for staining of endothelial cells, and shows membranous staining pattern. This study was undertaken to determine whether KBA.62 or SOX-10 could equal (or surpass) the sensitivity of S100 protein while offering superior specificity in the immunohistochemical evaluation of sentinel lymph nodes for metastatic melanoma.
Design: In this study we performed immunohistochemical stain for S100 protein, Sox-10 and KBA.62 on 50 lymph nodes with proven metastatic melanoma.
Results: SOX-10 detected all cases of metastatic melanoma (50 of 50 cases; 100%) compared to S-100 protein (48 of 50 cases; 96%) and KBA.62 (37 of 50 cases; 74 %). There was no "background" staining of normal cellular elements with SOX-10 or KBA.62. In contrast, S100 protein was expressed in scattered dendritic interdigitating reticulum cells in the paracortex of lymph nodes, showing cytoplasmic and nuclear positivity, sometimes posing significant difficulty in differentiating benign reticulum cells from single cell metastatic melanoma.
Conclusions: Our findings suggest that SOX-10 may be superior to S100 protein for identifying metastatic melanoma in a lymph node. KBA.62 was less sensitive than either marker, though more specific than S100 protein.

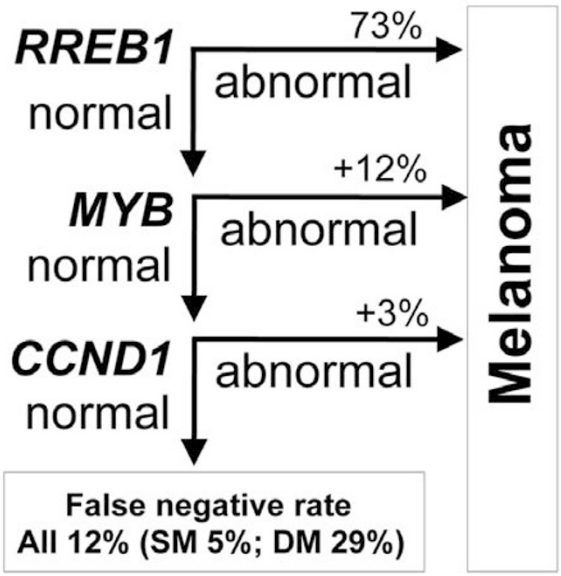
582 Performance Assessment of a Triple (RREB1/MYB/CCND1) Fluorescent In Situ Hybridization (FISH) Assay in Spindle-Cell and Desmoplastic Melanoma Argues for a Consecutive Testing Algorithm

SE Weissinger, BM Klaus, DN Silvers, P Moller, BAJ Horst, JK Lennerz. University of Ulm, Ulm, Germany; Columbia University Medical Center, New York, NY.
Background: Diagnostic confirmation of malignant melanoma is not always straightforward and two clinicopathologically distinct melanoma subtypes, desmoplastic (DM) and spindle cell melanoma (SM), can be especially challenging. While test performance of a recently established triple-probe FISH assay is striking in conventional melanoma, the diagnostic sensitivity in SM/DM has not been specifically addressed. Here we examined a large series of SM/DM for *RREB1/MYB/CCND1* abnormalities.
Design: Archival searches at two institutions identified a series of 33 SM/mixed/

DM cases. Hybridizations were performed using commercially available *RREB1/MYB/CCND1* probes and established diagnostic cutoffs. For literature review of test performance in conventional melanoma we performed pubmed searches in combination with manual review of references and tabulated the number of abnormal vs. tested cases for each probe and overall sensitivity comparisons. Routine test performance measures were calculated and statistical significance was defined as P<0.05.

Results: We performed a total of 123 hybridizations in 15 SM, 4 mixed and 14 DM cases. The assay was overall 88% sensitive (n=29 true positives). Although the sensitivity in DM was substantially lower (10/14=71% DM whereas 18/19=95% SM or 304/360=84% conventional melanoma), the differences did not reach statistical significance (*P*-range=0.14-1.0; Chi-square). Sensitivity by individual probesets was *RREB1* (24/32=75%), *MYB* (10/27=37%) and *CCND1* (6/29=21%). Due to the relatively high sensitivity of *RREB1*, our results indicate that a consecutive FISH-testing algorithm (Figure 1) can drastically reduce the number of hybridizations (i.e., from n=123 to n=57).

Conclusions: The triple FISH assay employing *RREB1*, *MYB* and *CCND1* probe sets is highly sensitive (88%) in SM/DM. We provide evidence for a practically efficient consecutive testing algorithm (Figure 1). Notably, the relatively high false negative rate in DM underscores the need for an additional reliable confirmatory melanoma assay and emphasizes the biological differences in this melanoma subtype.



583 HMGA2 Is a Reliable Immunohistochemical Marker for Separating Melanoma from Nevi

GG Yang, A Minasyan, M Lacey, J Gordon, T Knoll. Cell Marque Corporation, Rocklin, CA.

Background: Morphologically distinguishing malignant melanoma from nevi, benign or dysplastic can be a very challenging task. Current immunohistochemical (IHC) markers are not reliable for separating malignant melanoma from nevi based upon their cytoplasmic/nuclear staining patterns. High-mobility group AT-hook 2 (HMGA2) is a non-histone nuclear binding protein and an oncofetal protein, which is overexpressed in embryonic tissue and many malignant neoplasms, including ovarian cancer, but rarely in normal tissue. A study of this IHC marker was conducted for the identification of melanoma and differentiation from nevi.

Design: Surgical specimens of 38 cases of nevi and 47 cases of malignant melanoma were included in the study. One whole-slide section from each case was stained with monoclonal anti-HMGA2. Staining intensity was scored as 0 (negative), 1-2 (weak), 3 (moderate), 4 (strong); the labeling extent was tabulated as 0 (less than 5% positive cells), 1 (5-25% positive cells), 2 (26-75% positive cells), and 3 (greater than 75% positive cells).

Results: Of 47 cases of malignant melanoma, HMGA2 was expressed in 30 cases (63.8%) with a nuclear staining pattern. Of the 30 positive cases, 8 presented strong staining and greater than 75% positive cells; 13 cases showed moderate staining and 26-75% positive cells; and 9 cases demonstrated weak staining and 5-25% positive cells. Of 38 cases of nevi, only 2 cases (5.2%) were positive with weak staining and less than 50% positive cells.

Conclusions: HMGA2 is a useful marker for melanocytic lesions and can be used in differentiation of malignant melanoma from nevi.

584 The Photoprotective Effect of American Ginseng on Ultraviolet Radiation Exposed Skin

Q Zhang, J Ma, LM Pereira, Y Hou, E Lui. Schulich School of Medicine and Dentistry, Western University, London, ON, Canada; Western University Canada, London, ON, Canada.

Background: Solar damage of the skin is mainly due to the ultraviolet radiation (UVR) exposure. UVR plays important roles in both skin aging and skin carcinogenesis, which are mostly secondary to chronic UVR exposure. Depending on the wavelength, UVRs penetrate into human skin differently: UVA penetrates to the papillary dermis, while the UVB reaches only the epidermis. The immediate effects of UV exposure include tanning, sunburn and porphyria cutanea tarda, which presents as blistering at sun-exposed skin, especially hands and face. In this study, we are exploring the protective effects of American Ginseng (Panax quinquefolius) extract on UVB induced skin damage in hairless mice.

Design: The acute UV-induced skin injury was examined in adult female hairless mice (SKH-1) 24 hr after UVB (300mJ/cm^2) exposure. Ginseng extracts were topically applied on the backs of the mice. Mice were sacrificed 24 hours after UV exposure. Skin from the back and blood samples were collected. Histological analysis and cytokine quantification were performed from samples collected.

Results: UV exposure induced similar skin damage as seen in human skin, including epidermal vacuolar alteration, vesiculobullous changes at dermal-epidermal junction, disorganized stratum basale, and necrosis. Minimal acute inflammation was identified. Topical application of ginseng extract on the backs of the mice for three consecutive days prior to UV exposure provided significant protective effect with no or less severe vesiculobullous changes. Skin tissue cytokine analysis revealed that UV radiation resulted in an increase in TNF and IL-10, but not IL-1β levels. Ginseng pretreatment reduced cytokine response to UV exposure.

Conclusions: Our data suggest that American Ginseng extract showed protective effects against UV induced acute skin damage. (Funding from Ontario Research Fund, Ministry of Research and Innovation).

Education

585 Editorial Board Membership of Pathology Journals: A Social Network Analysis

Y Akdas, GA Barkan. University of Florida, Gainesville, FL; Loyola University Healthcare System, Maywood, IL.

Background: In the world of academic medicine where publication is an essential part of the job description, editorial board members of the journals–the gatekeepers of knowledge–carry a very important role. The selection process of these individuals is mostly not transparent. There may be overlap amongst the editorial boards, which may influence publication type, quality etc. The aim of this study is to identify the editorial board members of the most commonly read general (nonsubspecialty) pathology journals and assess the overlap among them.

Design: Top 40 Pathology journals with the highest impact factor in 2013 were identified. Journals that are mostly publishing basic science articles or specialized pathology (e.g. neuropathology, cytopathology etc.) were excluded from the study. The editorial board member lists of individual journals were identified from the journals' websites. Data collection and analysis were conducted in Excel, Netdraw and UCINET with two–mode affiliation data.

Results: The data set is composed of 13 journals (Table) and 1173 individuals who are editorial board members of these journals. 285 of 1173 (24.3%) individuals are editorial members in multiple journals (range: 2–8). Affiliation data were mapped in the Figure, where red dots depict the individuals with≥ 3 editorial affiliations and blue dots depict sampled journals.

Pathology Journals and Impact Factors

No	Journal Name	Impact Factor
1	Journal of Pathology	7.585
2	Modern Pathology	5.253
3	American Journal of Surgical Pathology	4.868
4	American Journal of Pathology	4.522
5	Laboratory Investigation	3.961
6	Advances in Anatomic Pathology	3.412
7	American Journal of Clinical Pathology	2.881
8	Histopathology	2.857
9	Human Pathology	2.843
10	Archives of Pathology and Laboratory Medicine	2.781
11	Virchows Archive	2.676
12	Pathology	2.657
13	Journal of Clinical Patholoy	2.435

Conclusions: Majority of the individuals (75.7%) hold a single editorship; however, a quarter of the individuals hold at least 2 editorships. There is a high interconnectedness among the journals. The overlap of the editorial boards is not associated with the impact factor; however, the journals which have more overlap are more commonly read among pathologists. There is more overlap among the journals that publish articles with similar content.