	Total Pap test No	LSIL No	%
<20	14343	517	3.6
20-29	336284	6902	2.05
30-39	656239	11346	1.73
40-49	705667	12651	1.79
50-59	265011	3542	1.34
≥60	70170	834	1.19
unknown	158874	2103	1.32
Total	2206588	37895	1.72

	HC2 HPV Positive			HC2 HPV Negative		
	Follow-up	CIN2+*(%)	CIN1	Follow-up	CIN2+*(%)	CIN1
<20	24	2 (8.3)	16	3	0 (0)	3
20-29	367	49 (13.4)	284	81	3 (3.7)	68
30-39	440	92 (20.9)	298	135	15 (11.1)	100
40-49	379	75 (19.8)	263	132	10 (7.6)	102
50-59	68	14 (20.6)	46	28	1 (3.6)	18
≥60	14	1 (7.1)	6	2	0 (0)	2
unknown	42	5 (11.9)	34	12	3 (25)	8
Total	1334	238 (17.8)	947	393	32 (8.1)	301

Conclusions: This is the largest case study in women with LSIL in China. The LSIL rate and HPV positive rate, and follow-up results were within the benchmark ranges for international cytology laboratories. A surprising finding is that 8.1% LSIL/HPV- women had histological CIN2/3, which is not supportive of HPV test as primary cervical cancer screening. These data may contribute to establishing a baseline for better understanding the status of cervical screening in China.

489 ASCUS Cervical Cytology Report Rate and Histological Follow-Up Finding in China's Largest CAP-Certified Laboratory

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Background: Data about ASC-US women with hrHPV testing and the histological follow-up is very rare in China.

Design: A retrospective study identified Paps between 2011 and 2015 from the large cytology laboratory. ASC-US cases with hrHPV test and histological follow-up results were recorded.

Results: A total 2,206,588 Pap tests were performed during the study period. Overall ASC-US report rate was 3.8%, with 5.6% in SurePath, 4.6% in LITUO, 4.1% in ThinPrep, 3.8% in LPT, and 2.3% in conventional Paps. ASC-US report rate was highest for women aged 40-49 years and declined with age. Of 18,574 women with HC2 testing, 6498 were HPV positive (35.0%). HPV positive rate was the highest in younger women (60.1%, <20 years), gradually declined with increased age for women aged <50 years, except for an increased curve in women 60 years. 6012 patients had histological results within 6 months after ASC-US. Overall CIN2 and severe lesions (CIN2+) were identified in 7.9% patients. 887 ASC-US/HPV+ women and 1022 ASCUS/HPV- women had histological results with 6 months after Pap/HC2 testing. CIN2+ lesions were detected in 14.0% ASCUS/HPV+ women including 35 (4.0%) squamous cell carcinomas (SCC), and 2.8% ASCUS/HPV- women including one SCC. CIN2+ lesion rates were no statistically different among different age groups.

Conclusions: This is the largest case study in ASCUS in China. The ASCUS rate and HPV positive rate, and follow-up results were within the benchmark ranges for international cytology laboratories. 2.8% ASC-US/HPV-women had CIN2/3 detection within 6 months, which was higher than that from thereports in West countries. These data can contribute to establish a baseline for cervical screening in China.

	HC2 HPV positive			HC2 HPV negative		
	Follow-up No	CIN2+(%)	CIN1(%)	Follow-up No	CIN2+(%)	CIN1(%)
<30	205	28(13.7)	136(66.3)	138	4(2.9)	62(44.9)
30-49	588	83(14.1)	360(61.2)	743	22(3.0)	390(52.5)
≥50	71	10(14.1)	45(63.4)	118	3(2.5)	57(48.3)
Unknown	23	3(13.0)	15(62.5)	23	0	11(47.8)
Total	887	124(14.0)	556(62.7)	1022	29(2.8)	520(50.9)

490 Diagnosis "Non-Invasive Follicular Thyroid Neoplasm with Papillary-Like Nuclear Fetaures" (NIFTP): Implications on the Risk of Malignancy (ROM) in the Bethesda System for Reporting Thyroid Cytopathology (TBSRTC)

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Background: NIFTP was recently proposed as a specific histopathologic diagnosis based on a set of reproducible morphologic criteria. Retrospective analyses suggest that this diagnosis will affect the ROM mostly in indeterminate throid Bethesda categories. In this multi-institutional retrospective study we report the potential impact of NIFTP diagnosis on the associated ROM for each TBSRTC category.

Design: A retrospective analysis was carried out on thyroid FNA cases with histologic follow up in three academic center hospitals (Institution A, B, & C), from January 2014 to December2015. Slides for all cases diagnosed as follicular variant of papillary thyroid carcinoma (PTC) specimens were reviewed and cases qualifying as NIFTP were separated from other cases of PTC.

Results: The case cohort included a total of 2185 resected thyroid nodules diagnosed on FNA as. Non-diagnostic -3.1%, Benign-34%, atypia/follicular lesion of undetermined significance (AUS/FLUS)-18.8%, follicular Neoplasm / suspicious for follicular neoplasm (SFN/FN)-19.5%, suspicious for malignancy (SM)-8.1% and malignant (M)-16.8%. On the surgical pathology follow-up, 1464 (67%) cases were diagnosed as benign, 628(29%) as malignant and 93 (4%) can be classified on re-review as NIFTP. The number of NIFTP cases was highly variable among all three institutions; -institution A-9%, institution B-4% & institution C-1%. The average ROM for all TBSRTC categories with NIFTP for institution A-45%, B-44%, and C-34.5%; and without NIFTP was institution A-35%, B-43%, and C30.1%.

Conclusions: Adoption of NIFTP terminology and institutional frequency of this diagnosis, would largely affect the rate of malignancy associated with specific FNA diagnosis, especially among the TBSRTC indeterminate categories. Therefore, providing a range for risk of malignancy for each TBSRTC diagnostic category is very much reflective of inherent limitations of diagnostic thresholds and interobserver variabilty in the diagnosis of thyroid lesions.

491 Abstract Withdrawn

Dermatopathology

492 Non-V600E BRAF Mutations Are More Common in Cutaneous Melanomas of Head and Neck and Upper Extremity

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Background: Approximately 50% of cutaneous melanomas have activating BRAF mutations, with 90% of these harboring the V600E mutation. FDA-approved anti-BRAF therapies are mainly available to treat V600E mutations and currently have limited use for treatment of other variants. This study assessed the frequency and clinical presentation of V600E and non-V600E BRAF mutations in head and neck which reported to have poorer clinical outcome compare to cutaneous melanoma of the other anatomic site.

Design: A retrospective review of the molecular genomic pathology results was performed. 67 cutaneous melanoma cases were identified between 2013-2016. For 55 cases, BRAF mutation status was determined by a next generation sequencing based panel and 12 cases were tested by Sanger sequencing.

Results: Sites of involvement included the head and neck (21/67), upper extremity (14/67), lower extremity (15/67), and trunk (11/67) with BRAF mutations identified in 36/67 (54%) of cases. BRAF V600E/K/R, L597S, L584F, and S467L mutations were detected in these cases. The distribution of BRAF mutations differed by anatomic site as shown in table1.

Anatomic Site	Total BRAF Positive N (%)	V600EN (%)	V600K N (%)	V600RN (%)	L597SN (%)	L584FN (%)	S467LN (%)
Head and Neck	8/21 (38%)	2/8 (25%)	2/8 (25%)	1/8 (12.5%)	1/8 (12.5%)	1/8 (12.5%)	1/8 (12.5%)
Upper Extremity	9/14 (64%)	2/9 (22%)	6/9 (67%)	0	0	0	1/9 (11%)
Lower Extremity	4/15 (26%)	3/4 (75%)	0	1/4 (25%)	0	0	0
Truncal	11/17 (65%)	10/11 (91%)	1/11 (9%)	0	0	0	0

Conclusions: For the first time we are reporting that cutaneous melanomas of the head and neck harbor a wider variety of BRAF alterations other than the classic V600E mutation. In addition the majority of the upper extremity cases have a V600K mutation. The wide array of BRAF mutations present in the head and neck compared to the other three anatomic sites may result from greater UV exposure.

493 Detection of Metastatic Melanoma Using Immunohistochemical Stains: How Many and Which Ones?

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Background: Despite the importance of the detection of metastasis in sentinel lymph nodes (SLNs) in cases of melanoma, there is no consensus regarding the markers that should be used. Protocols for histopathologic evaluation of SLNs vary among institutions. We present our experience in handling sentinel lymph nodes for the last 3 years in order to ascertain how many markers are sufficient for detection and which ones performed best. This has potential implications for the added cost and reimbursement patterns for SLN evaluation.

Design: A computer-based search of our files spanning the period from January 2013 to April 2016 revealed 228 cases of wide local excision of melanoma with biopsied SLNs. The protocol at our institution for handling formalin-fixed SLNs is to bisect the lymph node and section the entire node at 1 to 2 millimeter intervals. Three levels are stained with H&E and if found to be morphologically negative, all the sections are stained for S100, MART-1 and HMB45 by immunohistochemical (IHC) methods. The detection

of a single melanoma cell by either by H&E or IHC staining is sufficient to classify the lymph node as positive for metastatic melanoma. All the cases were reviewed for the presence of metastasis and capsular nevi.
Results: In our cohort of 228 cases, 19.73% of the cases were positive on H&E for metastatic melanoma (n=45). Out of the 183 negative cases on H&E, 4.9% (n=9) were positive by IHC. MART-1 was positive in 100% (n=9) of the positive cases, S100 in 77.78% (n=7) and HMB45 in 55.6% (n=4) of stained samples. The sensitivity and specificity of HMB45 were 55.56% and 88.89%, respectively. Capsular nevi were present in another 9 cases, comprising 4.9% of the SLNs. All of them (100%, n=9) stained positive for MART-1, 66.67% (n=6) for S100 and 11% (n=1) for HMB45. Only one case of spindle cell melanoma had positive SLNs that were detected on H&E stain.
Conclusions: Our results show that 4.9% of the positive sentinel lymph nodes were either negative or equivocal on H&E but positive on IHC. IHC stain panels are typically employed to balance sensitivity and specificity. MART-1 proved to be the most sensitive marker in our study, followed by S100. No cases of spindle cell melanoma required IHC. Our results support previous studies regarding the pattern of staining in capsular nevi and confirm the sensitivity of MART-1 in IHC staining for capsular nevi, and the high specificity (88.89%) and low sensitivity (55.56%) of HMB45. A larger study is needed to validate the utility of other markers such as MITF or SOX10 in detecting occult melanoma in SLNs.

494 Cutaneous Kaposi Sarcoma in Non-AIDS Related Immunosuppressed Patients: A Histologic Study of Morphologic Spectrum

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Background: Kaposi sarcoma (KS) is a low-grade vascular tumor associated with human herpesvirus 8 (HHV8) infection. It is known to be associated with the acquired immune deficiency syndrome (AIDS); however, it can also be seen in other settings such as immunosuppressed patients due to medications. Several histologic variants have been described such as patch lesions with thin slit-like vascular spaces (SLV-pattern), telangiectatic, ecchymotic, pyogenic granuloma-like (PG-like), lymphangioma-like and others. Studies looking into the morphologic spectrum of cutaneous KS in non-AIDS related immunosuppressed patients are limited.
Design: 32 specimens of cutaneous KS from 28 non-AIDS related immunosuppressed patients were identified in our archival material. All patients were seronegative for HIV. The morphologic details were evaluated by one dermatopathologist/soft tissue pathologist and one soft tissue pathologist blinded to the clinical background.
Results: Three patient groups were identified including (a) transplanted patients (14/28; 50%): 13 renal and 1 lung, (b) autoimmune diseases (9/28; 32%): 2 pemphigus vulgaris, 2 psoriasis, 1 bullous pemphigoid, 1 antisynthetase syndrome, 1 dermatomyositis, 1 systemic lupus erythromatosus and 1 Goodpasture syndrome, (c) malignancies (5/28; 18%): 1 diffuse large B cell lymphoma, 1 multiple myeloma, 1 Hodgkin lymphoma, 1 marginal zone lymphoma and 1 prostate carcinoma. In group (a), SLV-pattern was observed in (6/16; 38% biopsies), telangiectatic pattern in (4/16; 25% biopsies), glomeruloid pattern in (2/16; 13% biopsies) and each of lymphangioma-like, nodular, hyperkeratotic and ecchymotic in (1/16; 6% biopsies). In group (b), telangiectatic pattern was seen in (5/9; 55% biopsies), SLV-pattern in (2/9; 22% biopsies), PG-like in (1/9; 11% biopsies) and nodular in (1/9; 11% biopsies). In group (c), SLV-pattern was seen in (4/7; 57% biopsies) and telangiectatic pattern in (3/7; 43% biopsies).
Conclusions: In this series, the most commonly affected group of non-AIDS related immunosuppressed patients is post-organ transplant patients followed by patients with autoimmune diseases then patients with malignancies. The hematolymphoid malignancies were more common pre-exiting tumors than others. The most common histologic patterns in all groups were SLV-pattern and telangiectatic pattern with a slight predominance of the former in transplanted patients and patients with malignancies, possibly due to biopsying earlier lesions in these two groups of patients.

495 Genetic and Epigenetic Alterations of *Telomerase Reverse Transcriptase* (*TERT*) in Metastatic Melanoma

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Background: Reactivation of telomerase, the enzyme that maintains the integrity of chromosomal ends in proliferating cells, is critical for malignant transformation. *TERT* encodes the rate-limiting subunit of telomerase and, accordingly, is upregulated in 85%–90% of cancer cell lines and tissues. However, several genetic and epigenetic alterations are linked with this upregulation. In the case of melanoma, specific activating mutations in the *TERT* promoter are found in ~70% of metastatic tumors and less commonly in primary tumors. What alterations occur in the remaining 30% of cases is not well understood. Some possibilities include gene amplification or structural re-arrangements of *TERT,* or hypermethylation of the *TERT* promoter.
Design: The study cohort consisted of 76 patients with metastatic melanoma: 61 non-acral, 10 acral, and 5 mucosal primaries. Samples were screened for *TERT* promoter mutations by Sanger sequencing, and for *TERT* structural rearrangements and copy number variation by fluorescence *in situ* hybridization. *TERT* promoter hypermethylation was determined by MassARRAY and combined bisulfite restriction enzyme analysis. Activating mutations in *NRAF* and *BRAF* were also screened by Sanger sequencing.
Results: Primary tumors were from 40 females and 36 males, aged 16–93 years (median, 58 years). *NRAS*, *BRAF*V600, and *TERT* promoter mutations were found in 18 (24%), 36 (47%), and 53 (70%) samples, respectively. *TERT* structural rearrangements were found in 7 (9%) samples (4 acral, 2 non-acral, 1 mucosal/vulvar) and commonly occurred alongside promoter hypermethylation (n=6) or gene amplification (n=2), but were mutually exclusive of promoter mutations. Promoter hypermethylation occurred in 44 (58%) samples, commonly alongside promoter mutations (n=25), or in the absence of any other alteration (n=13; 17%).
Conclusions: Several genetic and epigenetic alterations that are linked with *TERT* upregulation are found in metastatic melanocytes. Prominent amongst these are promoter mutation and hypermethylation. Structural rearrangement of *TERT* is more predominant in acral than non-acral melanoma. Further studies are warranted on the relative prevalence of these alterations in primary melanoma, and the correlation of each alteration type with subsequent metastatic potential.

496 Genomic Alterations and Tumor Mutational Burden in Melanoma Subtypes

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Background: Metastatic melanoma (mMM) is one of the most targetable malignancies, with both kinase inhibitors and immunotherapies meaningfully extending the lives of patients with this disease. Different subtypes of melanoma harbor a variety of genomic alterations (GA) that indicate potential targeted and immunotherapy options.
Design: DNA (≥50ng) was extracted from 2,197 cases of relapsed, refractory and mMM. Comprehensive genomic profiling (CGP) was performed for up to 315 cancer-related genes on hybrid-capture, adaptor ligation-based libraries (mean coverage depth >600X). Tumor mutational burden (TMB) was calculated from a minimum of 1.11 Mb sequenced DNA and reported as number of mutations/Mb. The results were analyzed for all classes of GA, including base substitutions, insertions and deletions (short variants; SV), rearrangements, and copy number changes.
Results: Samples were divided into cutaneous (CT; 90%), acral lentiginous (AL; 1%), Spitzoid (SP; 1%), mucosal (MC; 2%) and ocular (OC; 5%) subtypes. CGP revealed characteristic genomic signatures within each group (Table). *BRAF* was mutatedin 38% of CT of which 55% were V600E and 25% V600D/K, and 20% were *BRAF* amplifications, fusions, or other missense mutations. *NF1* GA were common in CT and MC but rare in OC. , *KIT* GA were rare in CT but common in AL and MC. The proportion of patients with TMB in CT was extremely high (42% with >20 mut/Mb). The frequency of *BRAF* GA was lower in AL (18%) and MC (15%), and extremely low in OC (2%). SP GA were dominated by *BRAF* fusions (60%) and fusions involving other kinases. *KIT* GA were also prominent in MC. TMB for MC and OC mMM were extremely low, especially compared to CT.

	CT	AL	SP	MC	OC
Samples	1991	22	22	44	105
Significant-driver GA	BRAF (38%) NF1 (21%) PTEN (12%) KIT (5%)	BRAF (18%) NF1 (18%) PTEN (18%) KIT (18%)	BRAF Fusion (60%)ROS1 Fusion (3%)RET Fusion (3%) NTRK1 Fusion (1%)ALK Fusion (1%)	BRAF (15%) NF1 (32%) KIT (25%) PTEN (13%)	BRAF (2%)NF1 (2%)(GA in BAP1, GNAQ, GNA11 or MYC found in 100% of cases)
TMB>10 mut/Mb	61%	NA	NA	3%	3%
TMB >20 mut/Mb	42%	NA	NA	0	1%

Conclusions: Genomic profiles and TMB scores differ across mMM subtypes. By far the most frequent GA in CT and SP mMM involve *BRAF* and the high TMB in CT facilitates the selection of both targeted and immunotherapies for these patients. Although MC and OC have significantly lower BRAF GA frequency and lower TMB scores, targetable GA that provide therapeutic options for patients can be identified in these tumors.

497 Immunohistochemical Characterization of Fumarate Hydratase (FH) and Succinate Dehydrogenase (SDH) in Cutaneous Leiomyomas for Detection of Familial Cancer Syndromes

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Background: Hereditary Leiomyomatosis and Renal Cell Cancer (HLRCC) Syndrome is caused by germline mutations in the FH gene, resulting in marked disturbances of cellular metabolism. HLRCC is associated with increased incidence of leiomyomas and a potentially aggressive variant of renal cell carcinoma (RCC). Early detection of HLRCC provides a rationale for serial renal imaging, which might reduce mortality by detecting RCC at an early stage. Absent immunohistochemical expression of FH has previously been employed to diagnose HLRCC-associated RCC, but immunohistochemical staining of leiomyomas is not standard practice.
Design: Our institutional database was searched for cutaneous leiomyomas diagnosed between 1995 and 2015. All H&E-stained sections were re-examined and relevant clinicopathological data was recorded. Since both FH and SDH are Krebs cycle enzymes and renal tumors of patients with HLRCC might demonstrate an oncocytic morphology (reminiscent of SDH-deficient RCC; USCAP 2016 abstract 1048), we performed immunohistochemistry (IHC) on whole sections from each case to evaluate for both FH and SDH-B expression. IHC slides were visually scored as negative or positive and IHC data was recorded in a blinded manner by two independent sources, with consensus reached in cases of discordance (based on a blind re-review performed by two pathologists in concert).

Results: Ninety-six cutaneous leiomyomas from 87 patients were identified; 12 of these specimens were from 6 patients with documented HLRCC, proven by mutational analysis performed either on the patient or an immediate family member. FH expression was negative in 9 specimens and retained in 85 specimens; two cases were equivocal with minimal FH expression. Seven of the 9 negative specimens were from patients with HLRCC, as were both of the equivocal specimens. The overall sensitivity and specificity of negative FH expression in leiomyomas for patients with HLRCC were 70.0% and 97.6%, respectively. Inclusion of cases classified as equivocal increased sensitivity to 75.0%. SDH-B expression was retained in 95 specimens and equivocal in 1 specimen.
Conclusions: Loss of FH immunohistochemical expression in cutaneous leiomyomas is a sensitive and specific marker for HLRCC syndrome, thus suggesting a role for prospective FH IHC in patients with these tumors to screen for HLRCC. Molecular sequencing could then be used to confirm genetic associations.

498 Merkel Cell Carcinoma Has a High Number of Neoantigens but Low PD-L1 Expression

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Background: Merkel cell carcinoma (MCC) is an aggressive neuroendocrine neoplasm of the skin with no effective therapies. MCC has been shown to have a high mutational burden; higher mutational load and increased numbers of neoantigens in melanoma and lung cancer has been shown to be strongly associated with a greater clinical benefit from immune checkpoint inhibitors. While PD-1/PD-L1 are involved in the mechanism of immune checkpoint blockade, PD-1/PD-L1 expression levels by immunohistochemistry has been an equivocal predictor of clinical response. We use the two established approaches—mutational analysis and PD-1/PD-L1 immunohistochemical analyses—to evaluate for how MCC might respond to immune checkpoint blockade
Design: We identified a set of 12 well-characterized MCCs that were analyzed by high coverage exome sequencing using 2x101bp reads. Sequence variants were called using VarScan2 and annotated using VEP. Four cases had paired normal blood allowing the direct identification of somatic mutations; for cases without paired blood, somatic mutations were inferred by removing common SNPs using public database. HLA type was computed from exome data using ATHLETES package. Neoantigens were estimated from somatic mutations using the pvac-seq pipeline based on NetMHC4.0 HLA class I binding predictions. Immunohistochemistry was performed for PD-L1 (SP142) and PD1 (NAT105) and scored in tumor cells.
Results: A mean of 572 (range 129-1007) somatic mutations/case predicted to alter protein coding were identified (21 mutations/Mbase). Seven of the 12 cases typed as HLA-A*02:01and could be further analyzed for neoantigens. A mean of 345 neoantigens were predicted in each case with a mean netMHC score fold change of 67.6 for predicted MHC class 1 nanomeres. Immunohistemistry for PD-L1 showed approximately 1-10% tumor cell staining in all cases; no cases had >25% positive tumor cells. PD1 was positive in rare cases.
Conclusions: Here we show that MCC harbors a high number of predicted neoantigens despite minimal reactivity for PD-L1 and PD1 by immunohistochemistry. While the immunohistochemical studies are not conclusive, previous studies show that PD-1/PD-L1 expression by immunohistochemistry is not a strong predictor of clinical response to checkpoint blockade. Given the high mutational burden in our samples, we are hopeful that MCC may be amenable to immune checkpoint therapy or tumor vaccines.

499 T-Cell Receptor (TCR) Gene Rearrangement Profiles in Skin Biopsy of Mycosis Fungoides

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Background: Due to overlap in clinical presentation and morphology of mycosis fungoides (MF) with other reactive lymphoid infiltrations, TCR clonality assays are helpful in confirming a diagnosis of MF. The standardized BIOMED-2 multiplex PCR protocol has been widely used for this purpose. TCR gene rearrangement pattern or genotypic profile has been studied in other mature T-cell neoplasms, however, such study is very limited in MF. We investigated the TCR gene rearrangement pattern on 24 well-characterized MF cases to determine the TCR gene segment usage and compared to other T-cell neoplasms.
Design: DNA was extracted by EZ1 DNA Tissue Kit (Qiagen) from formalin-fixed, paraffin-embedded (FFPE) skin biopsies of 24 MF patients. TCRG and TCRB assays were performed in duplicate using analyte specific reagents (InVivoScribe Technologies) and capillary gel electrophoresis according to BIOMED-2 protocol. Only those cases with detected clonal peaks were included in this study (22 for TCRG assay, 20 for TCRB assay).
Results: More than one clonal peaks were observed in most MF patients (Table 1). In 12/24 patients with sequential biopsies or skin biopsies from multiple sites, TCRG and/or TCRB clonal peak patterns were identical in 8 patients while the remaining 4 patient samples showed clonal evolution with additional peaks. For these 4 patients, TCR clonal results on the initial biopsy were included in this study. Compared to BIOMED-2 studies on mature T-cell neoplasms (Bruggemann M et al, Leukemia 21, 215, 2007), we observed similar preferential usage of Vγf1 but much higher usage of Vγ9 and Vγ10 in MF patients; also similar usage of Vβ-Jβ but higher usage of Dβ-Jβ region was seen (Table 1).

	Clonal peak(s) detected (case#)	TCR segment usage %
TCRG assay		
Vγ9 only	1	5%
Vγ10 only	2	9%
Vγf1 only	6	27%
	6	27%
	5	23%
	2	9%
Total	22	100%
TCRB assay		
Dβ-Jβ only	1	5%
Vβ-Jβ only	7	35%
	12	60%
Total	20	100%

Conclusions: Our study indicated that except for the Vγ11 region, all other Vγ regions, as well as Vβ-Jβ and Dβ-Jβ regions were frequently used by this group of MF cases. These findings are similar to those seen in other mature T-cell neoplasms; however, it appears MF may have more usage of Vγ9, Vγ10, and Dβ-Jβ regions. Further sequencing of TCR gene regions in MF might be required to determine the significance of the preferential usage of these TCR gene segments.

500 Primary Cutaneous Solitary Fibrous Fumor (SFT) – A Series of 23 Cases

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Background: While cutaneous SFT have been described, definitive diagnosis is difficult due to overlapping features with other superficial tumors. We describe the largest series to date of cutaneous SFT.
Design: SFTs from four institutions were reviewed. For inclusion, the SFT had to arise in subcutis or dermis, with diagnosis confirmed by expert review.
Results: 23 met criteria. Patients ranged from 16-80 years (mean: 48). There was a marked female predominance (18F; 5M). Tumors involved the head (9), including scalp (4), eyelid (2), lip (1), cheek (1), external ear canal (1), and the thigh (7), shoulder (2), back (2), upper arm (1), ankle (1), and great toe (1). Mean size was 2.84 cm (range, 1.0 - 7.0). The majority (n=18) had typical histologic features, predominantly on the cellular end of the spectrum, with irregular fascicles of spindled cells with staghorn-like vessels, and variable amounts of collagen. One had features consistent with previous criteria for malignancy: striking hypercellularity, mitotic rate of 5/10 HPF, and necrosis. In this case, areas with epithelioid cells and clear cell change reminiscent of PEComa were present. The shoulder lesion showed ovoid-to-spindled cells arranged in whorled and nested patterns with a paucity of ectatic vessels. One eyelid tumor was a giant-cell rich SFT. One lesion from the scalp contained cellular nodules with nuclear atypia, while another scalp lesion was predominately myxoid. The toe lesion had a prominent pseudovascular pattern with extravasated red blood cells. Tumor necrosis was evident in three cases (all < 25%). Mitotic activity ranged from 0 to 10 MFs/10 HPFs (mean: 2MFs/10 HPF). By immunohistochemistry, 15/16 were STAT6+; 19/19 were CD34+. The tumors were subclassified by risk stratification criteria proposed by Demicco et al: 18/20 low risk and 2/20 moderate risk, including the one previously classified as malignant SFT. Three could not be stratified due to lack of information on tumor size. Follow-up, available on 7 cases, showed no recurrence/ metastasis (mean follow-up 100 mos., range 2-241).
Conclusions: Cutaneous SFT are more common in women and most commonly involve the head. They are usually low risk by proposed criteria and appear to behave in an indolent fashion, though larger studies are needed to confirm this.

501 SP174 Antibody Lacks Specificity for NRAS Q61R and Cross-Reacts with HRAS-and KRAS- Q61R Mutant Proteins in Malignant Melanoma

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Background: HRAS, KRAS and NRAS, highly homologous proteins, are often mutationally activated in cancer. Usually, mutations cluster in codons 12, 13 and 61 and are detected by molecular genetic testing of tumor DNA. Recently, immunohistochemistry with SP174 antibody has been introduced to detect NRAS Q61R mutant protein. Studies on malignant melanomas showed that such an approach could be a valuable alternative to molecular genetic testing. This investigation was undertaken to evaluate the value of SP174 immunohistochemistry for detection of NRAS Q61R mutant isoform.
Design: Two hundred ninety-two malignant melanomas were evaluated using a rabbit monoclonal antibody, clone SP174 (Spring™ Bioscience, Pleasanton, CA), diluted 1:100 and Leica Bond-Max automated immunostainer (Leica, Bannockburn, IL). *NRAS* codon 61 was PCR amplified and evaluated using Sanger sequencing in 116 tumors.

Subsequently, cases (n=13) with unsatisfactory/questionable Sanger sequencing results were blindly evaluated using qPCR and Ion Torrent™ next-generation sequencing approaches.
Results: Twenty-nine tumors showed positive immunoreactivity with SP174 antibody. *NRAS* codon 61 was PCR amplified and sequenced in 24 positive and 92 negative cases. A c.182A>G substitution leading to NRAS Q61R mutation was identified in 22 tumors. Two *NRAS*-wild type tumors revealed c.182A>G substitutions in *H-* and *K-RAS* codon 61, respectively. Both mutations were detected by next-generation sequencing and independently confirmed by Sanger sequencing. None of 85 *NRAS* codon 61-wild type tumors and 7 *NRAS* mutants other than Q61R showed immunoreactivity with SP174 antibody.
Conclusions: SP174 antibody was 100% sensitive in detecting NRAS Q61R mutant isoform in malignant melanoma, but not fully specific as it cross-reacted with HRAS and KRAS Q61R mutant proteins. Therefore, pinpointing which *RAS* gene is mutated depends on molecular genetic testing. The rarity of H-and K-RAS Q61R mutants in malignant melanoma let previous investigations erroneously concluded that SP174 is specific for NRAS Q61R mutant protein.

502 Immunohistochemistry Reveals an Increased Proportion of c-Myc-Positive Cells in a Subset of Subcutaneous Panniculitis-Like T-cell Lymphomas

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Background: Subcutaneous panniculitis-like T-cell lymphoma (SPTCL) is a malignant primary cutaneous T-cell lymphoma that shares significant clinical, histopathologic, and immunophenotypic overlap with lupus erythematosus panniculitis (LEP).
Design: We performed immunohistochemistry for the c-Myc oncoprotein on 22 cases of SPTCL and 12 cases of LEP to evaluate if there are quantitative or qualitative differences in expression of this marker in these diagnostic entities. For each stained slide, we manually counted nuclei in at least three 40x fields that appeared to have c-Myc hotspots. In these fields, the percent of all nuclei and the percent of nuclei around adipocytes with at least dim c-Myc expression were determined. In addition, we performed fluorescence in situ hybridization performed on 4 cases of SPTCL with high c-Myc expression to determine if these cases showed evidence of *c-MYC* rearrangement or amplification.
Results: The percentage of all cells that were c-Myc positive in the 22 SPTCL cases ranged from 0.76% to 15.7%, with a mean of 5.0% and a median of 4.5%. In contrast, in the 12 LEP cases, the percentage of c-Myc positive cells in the cases ranged from 0.34% to 3.7%, averaged 1.4%, and the median was 0.81%. In SPTCL cases, the fraction of nuclei around adipocytes that expressed c-Myc averaged 7.7% and ranged from 0 to 23%, with a median of 6.0%. In contrast, in LEP cases, the mean percentage of Myc-positive nuclei around adipocytes was 2.1%, median was 1.4%, and this figure ranged from 0.39 to 6.9% ($p = 0.006$). We did not find a correlation between the percentage of c-Myc positive cells and the percentage of Ki-67 positive cells. In addition, c-Myc expression did not correlate with patient age. Our findings also suggest that a substantial number of c-Myc-positive cells with are CD8-positive T cells. FISH performed on 4 cases of SPTCL with a relatively high level of c-Myc expression showed no evidence of *c-MYC* rearrangement or amplification.
Conclusions: We find a significant difference in c-Myc expression in cases of SPTCL as compared with LEP. Our work demonstrates that c-Myc expression levels differ between these two histologic mimics and suggests that this marker may be useful in distinguishing cases of SPTCL with high c-Myc expression. In addition, the findings suggest heterogeneity of c-Myc expression within SPTCL cases. Additional work is necessary to validate these findings in an independent cohort.

503 PD-L1 Expression in Desmoplastic Melanoma Is Associated with Depth of Invasion, Cytotoxic Tumor-Infiltrating Lymphocytes and Mixed Subtype Cytomorphology

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Background: Desmoplastic melanoma (DM) is divided into two variants which differ in histopathology and biology, with the metastatic potential of the mixed DM (MDM) subtype surpassing that of pure DM (PDM). Recently, patients with metastatic DM have been shown to respond more favorably to anti-PD/PL1 therapy than other melanoma subtypes. Given this, we sought to evaluate PD-L1/2 expression in DM and to correlate these with subtype, CD8+ lymphocyte status, histopathologic prognosticators, and select genetic alterations.
Design: Of archival annotated samples retrieved, 86 (36 MDM, 50 PDM) met inclusion criteria and were immunohistochemically semiquantitatively evaluated. For PD-L1/L2, cases with 5% or more tumoral expression and for CD8, cases with a predominantly peri/intra-tumoral CD8+ infiltrate were scored positive. Univariate analysis (chi-square and Wilcoxon) identified potential confounders and a nested case-control study was accomplished using multiple logistic regression.
Results: Overall, for PD-L1; 49% of DM cases were positive and 71% of DM cases with thickness >4mm were positive; PD-L1 expression differed by median depth (3.29mm, IQR =3.58mm for PD-L1 positives vs. 1.75mm,IQR=2.04mm for PD-L1 negatives, p=0.0002) and was linearly associated with increasing depth of invasion (p=0.0003). PD-L1 positive cases were more likely to display CD8+ lymphocytes (60% vs 28% p=0.0047).The presence of CD8+ lymphocytes correlated with depth of invasion >1mm (p=0.022). No correlation was observed with *RETp* or *TERT* promoter mutations. On multivariate analysis, PD-L1 was 5.58x more likely to be expressed in MDM than PDM (p=0.0153), and CD8+ staining was 4.75x more likely in PD-L1 positive cases than PD-L1 negative (p=0.0205). PD-L2 expression was observed in 48% of cases but did not correlate with either PD-L1 or CD8+ expression, or any of the other variables analyzed.
Conclusions: In DM, correlation of tumoral PD-L1 with increased depth of invasion as well as presence of CD8+ lymphocytes implicates the tumoral immune microenvironment with advancing disease. Enhanced tumoral PD-L1 expression in MDM provides an insight into the differential pathogenesis of DM subtypes. MDM patients are likely better candidates for anti-PD/PD-L1 therapy.

504 SATB2 Expression in Merkel Cell Carcinomas: An Immunohistochemical Study of 165 Cutaneous Neoplasms

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Background: SATB2 (special AT-rich sequence-binding protein 2), a transcriptional regulator involved in osteoblastic and neuronal differentiation, is well recognized as a marker of osteoblastic and colorectal neoplasms. Additionally, a recent study has shown that it is also a sensitive marker for neuroendocrine tumors of hindgut origin. In this study, we investigated SATB2 expression in Merkel cell carcinomas and other various cutaneous tumors.
Design: Formalin-fixed, paraffin-embedded whole and tissue microarray sections from 64 Merkel cell carcinomas and 101 non-melanocytic skin tumors (77 adnexal tumors, 10 squamous cell carcinomas, 9 basal cell carcinomas, and 5 cutaneous hematolymphoid neoplasms) were obtained and immunostained for SATB2 (polyclonal, 1:250 dilution; Sigma). 5 cases of metastatic pulmonary small cell carcinoma were also included. Only nuclear staining was considered to be "positive".
Results: SATB2 nuclear positivity was seen in 35 of 64 (54.7%) Merkel cell carcinomas. In contrast, all cases of cutaneous adnexal tumors, squamous cell carcinomas, basal cell carcinomas, and hematolymphoid neoplasms were completely negative. 3 of 5 cases of metastatic pulmonary small cell carcinomas also stained positively with SATB2.
Conclusions: SATB2 expression occurs in a subset of Merkel cell carcinomas and awareness of this immunoreactivity is important to preclude significant diagnostic pitfalls with tumors in the differential diagnosis. Although it is not suitable to distinguish Merkel cell carcinomas from metastatic neuroendocrine carcinomas of pulmonary or gastrointestinal origin, there may be some diagnostic utilities of SATB2 expression in certain situations.

505 PD-L1 and c-MYC Status in Merkel Cell Carcinoma: An Immunohistochemical and mRNA Chromogenic *In Situ* Hybridization Study

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Background: Merkel cell carcinoma (MCC) is a rare cutaneous neuroendocrine carcinoma with few effective therapeutic options and with significant mortality rate. Programmed cell death 1 (PD-1) is an inhibitory receptor and plays a major role in tumor immune escape. PD-L1 is the ligand for PD-1 and is expressed on antigen presenting cells and lymphocytes, as well as nonimmune cells. The role of PD-L1 protein expression in MCC was previously investigated, but its significance remains controversial. Furthermore, recent studies have also documented frequent c-MYC overexpression in MCC. Prompted by these observations, we analyzed our archival, well-characterized MCC cases for PD-L1 and c-MYC expression by immunohistochemistry (IHC) and mRNA chromogenic *in situ* hybridization (CISH).
Design: 68 cases (61 cases for PD-L1 IHC; 20 cases for *PD-L1* mRNA CISH; and 17 cases for *c-MYC* mRNA CISH) were available for tissue microarray construction. PD-L1 (pre-diluted, SP263, Ventana) IHC was performed according to the manufacturer's instructions. mRNA *PD-L1* and *c-MYC* CISH were performed using the RNAscope® 2.0 FFPE Assay (ACD Inc. Hayward, CA). Follow-up information was obtained from our medical records. Possible correlations with a wide variety of clinicopathological variables were evaluated using the chi-squared test.
Results: By IHC, 28/61 (45.9%) cases showed PD-L1 protein expression (tumor-infiltrating immune cells only: 27 cases; both tumor cells and tumor-infiltrating immune cells: 1 case). By CISH, *PD-L1* and *MYC* mRNA expression was present in 4/20 (focal, tumor cells and tumor-infiltrating immune cells) and 13/17 (tumor cells) MCC, respectively. Follow-up information was available in 30 cases. PD-L1 and MYC expression was not correlated with any of the evaluated clinicopathological parameters, including outcome.
Conclusions: In our study, almost all MCC cases showed a PD-L1 protein expression only in the tumor-infiltrating immune cells. This significant discrepancy from the previous studies highlights the importance of antibody selection. It may also reflect that levels of *PD-L1* mRNA are too low to be detected by our IHC method. Alternatively, some PD-L1 protein expressed tumor cell populations were missed by sampling bias. Finally, we also confirmed a high frequency of *MYC* mRNA expression in MCC and MYC may play a role in MCC tumorigenesis as well as potential therapeutic targeting.

506 Expanded Traditional Melanoma FISH Testing versus CAP-QPCR to Identify High-Risk Melanocytic Lesions

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Background: Borderline melanocytic tumors (BMT) are a heterogeneous group of lesions that are diagnostically challenging. Although histopathology remains the gold standard for diagnosing melanoma, adjuvant tools are often required to make a definitive diagnosis. One such tool that has been widely used for diagnosing BMT is fluorescent

in-situ hybridization (FISH). In addition, cell adhesion phenotyping by quantitative PCR (CAP-QPCR) was shown to identify high-risk melanocytic tumors at risk of regional metastasis at diagnosis. Here, our aim was to compare FISH and CAP-QPCR for their ability to identify high-risk BMT at diagnosis.

Design: 44 patients with FISH-tested BMT (from 2013-2015) were identified in archive. 14 of 44 BMT were interpreted as melanoma which eventuated in 11 sentinel lymph node (SLN) biopsies. The remaining non-melanoma BMT were MELTUMP (n=4; one with SLN biopsy), atypical compound or dermal nevi (n=16), atypical Spitz nevi (n=9) or atypical deep penetrating nevus (n=1). FISH testing was performed on formalin-fixed, paraffin embedded (FFPE) tissue with the established enumeration criteria for melanoma diagnosis, including probes targeting *RREB1, MYB, CCND1, MYC* and *CDKN2A* loci. CAP-QPCR testing was also performed in all cases as follows: RNA was extracted from FFPE sections, reverse transcribed and further analyzed by array-based QPCR. The test was considered positive if the ITLP gene signature, i.e. expression of *i*ntegrin β3, *t*issue-type plasminogen activator, *l*aminin B1 and tumor antigen *p*53 mRNA was detected at previously established cut points.

Results: 1/30 non-melanoma BMT were FISH-positive (97% specificity). 12/14 melanomas were FISH-positive (86% sensitivity). As expected, sensitivity and specificity of CAP-QPCR – a test intended to identify high-risk melanoma – showed higher specificity (100%) and lower sensitivity (50%) as only a subset of melanomas are high-risk. In line with these data, the prognostically important Breslow thickness (p=0.0016), mitotic rate (p=0.015) and SLN positivity (p=0.028; Table) were associated with CAP-QPCR positivity but not FISH positivity as determined by Wilcoxon or Fisher exact test.

Conclusions: Overall, FISH can be used as an initial ancillary method to aid with histopathologic impression, while CAP-QPCR can predict the biological behavior and guide treatment and surveillance of BMT. Larger scale studies are necessary to further validate these observations.

507 Diagnosis of Spitz Tumors in Children and Adults Using Artificial Neural Networks

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Background: Spitz Tumors (ST) are infrequent melanocytic tumors. Their classification in Spitz Nevus (SN) or Atypical Spitz Tumor (AST) depends on histopathological features and immunohistochemical markers. Artificial Neural Network (ANN) is an Artificial Intelligence (AI) tool that can interpolate results considering past experiences. This work is about the influence of age on these diagnoses via an ANN, and is part of a larger study about the possible contribution of AI in Spitz Tumors classification.

Design: An ANN is composed of "neurons" distributed over different layers. Each neuron of a layer is connected to all the neurons of the next layer through a weighted arc. The ANN training consists in computing the weights of the arcs, using a training data set composed of the input values and the expected ouput values. Then, the ANN can interpolate any result from any unknown input value.

We analyzed 40 ST (33 SN and 7 AST) on 11 morphological features (diameter, thickness, cytonuclear atypia, subcutis extension, asymmetry, poor circumscription, pagetoid spread, lymphocytic infiltrate, high cellular density and Kamino bodies) and used 4 immunomarkers (Melan-A, HMB-45, BRAFV600E and ALK).

We have trained and tested ANNs composed of 15 inputs (the data previously mentioned) and one input (SN or AST). The data have been split in 4 subsets in order to perform a 3-cross over validation.

Results: ANNs trained on adult tumors classified correctly 78% of adult tumors and 82 to 91% of children tumors. However, ANNs trained on children tumors gave correct diagnoses for 66 to 78% of adult tumors and 72 to 82% of children tumors.ANNs trained on adult tumors gave more reliable diagnoses than ANNs trained on children tumors.

Conclusions: This study using an AI tool on a small cohort doesn't allow to distinguish NS from TSA with certainty on histopathological and immunomarkers data. But it strengthens the idea that adult tumors is a better base than children tumors for Spitz tumors diagnosis.

508 Diagnosis of Spitz Tumor Using Artificial Neural Networks

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Background: Spitz tumors (ST) are unusual melanocytic tumors classified in Spitz nevus (SN) or Atypical Spitz tumor (AST) according to histological and immunochemical non-consensual criteria.

Artificial Neural Network (ANN) is an Artificial Intelligence (AI) tool that can interpolate results considering past experiences.

We aim at studying the ability of ANN to diagnose SN, AST and melanoma.

An ANN is composed of neurons . Each neuron of a layer is connected to all the neurons of the next layer through a weighted arc. The ANN training consists in computing the weights of the arcs, using a training dataset composed of the input values and the expected output values , ANN interpolate any result from any unknown input value

Design: We have analysed a set composed of 38 SN, 8 AST and 20 melanomas, according to 18 morphological characteristics (age, diameter, thickness, cytonuclear atypia, subcutis extension, asymmetry, poor circumscription, pagetoid spread, lymphocytic infiltrate, high cellular density, Kamino bodies, irregular and confluent nests, deep mitosis, Grenz zone infiltration, localization, ulceration, mitotic rate, atypical mitosis) and 4 immunohistochemical markers (Ki67, p16, BRAFV600E and ALK).

We have trained and tested ANNs composed of 22 input values (the values previously mentioned) and one output (SN, AST or melanoma). Data have been split in 5 subsets in order to perform a 5-cross validation.

Results: ANNs classified correctly 77% of the tested cases. They diagnosed correctly 70% of the SN cases, 60% of the AST cases and 96% of the melanoma cases. Hence, ANNs clearly distinguished melanoma from ST. We can remark that the worst results correspond to the least represented cases of the dataset (only 8 AST cases).

ANNs can classify SN, AST and melanoma. Indeed, the chosen input parameters allowed ANNs to distinguish melanoma from SN and AST. Actually, the fact that there were only 8 AST cases in the entire dataset influenced the ANNs' ability to distinguish SN from AST. We now aim at increasing the number of AST cases in particular and also to take into account this disequilibrium of the dataset, in order to improve the ANNs classifications.

Conclusions: We studied the possibility to use an ANN in order to classify SN, AST and melanoma cases. The results prove that ANN can distinguish correctly 79% of the cases of our dataset. Further work will consist in increasing the number of cases of the less represented category and to study solutions in order to increase the ANN accuracy and the dataset imbalance.

509 Antiviral Drugs as a Therapy Strategy in Advanced Stage of Malignant Melanoma?

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Background: Skin cancer is the second most common cancer in the Netherlands with malignant melanoma (MM) as the major cause of death. In advanced disease, molecular profiling has led to the development of targeted therapies. However, a multitude of critical issues have arisen with these new targeted therapies: I) development of drug resistance, II) considerable adverse effects and III) unsolved economic issues reflecting the high costs of these therapies. Finally, adequate treatment options for the remaining 52% MM patients harbouring of non-mutated BRAF are lacking.

Design: In the framework of drug repositioning, establishment of new drug combinations and patient stratification we have conducted an *in-vitro* investigation with human MM cell lines, assessing the tumour-sensitivity to approved systems medicine including the BRAF-inhibitor Vemurafenib, classical chemotherapy Dacarbacine and the immunomodulator Interferon alpha-2b as wells as off-use antiviral drugs Ribavirin, Saquinavir and Cidofovir. The selected cell line panel consisted of BRAFV600 mutated (SKMel28, SKMel2, IGR-1) and BRAF wild-type (SKMel2, MEWO) MM cell lines. Cell viability was measured with a MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium-bromide) assay.

Results: Our experiments showed heterogeneous sensitivity to systems medicine alone and in combinatory designs with antiviral drugs. The different drug-responsiveness yielded into two drug-related groups revealing a Ribavirin and Cidofovir sensitive group and a less sensitive group. In line with these findings, cytomorphological observations, *in-vitro* characteristics and with one exception also the BRAF mutational status could be clustered to the identified drug-related groups. Distinct combinatory treatments of approved medicine with off-use antiviral drugs showed significantly reduced tumor cell viability, when compared to monotherapy or non-treated control.

Conclusions: We could show responsiveness of antiviral drugs and selected drug combinations in the investigated MM cell lines. Stratification based on drug-responsiveness, morphology and *in-vitro* characteristics could be developed. The underlying molecular profile of the drug-related groups remains to be elucidated in future experiments. Cell line modelling represents a tool for personalizing treatment strategies in MM patients by identifying BRAF-inhibitor responders and those benefiting from combination therapy or alternative drug strategies.

510 Utility of a Next-Generation Sequencing Panel in Myeloid Cutaneous Lesions: A Novel Approach in Distinguishing Leukemia Cutis from Nonneoplastic Myeloid Infiltrates

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Background: Cutaneous infiltrates seen in leukemias can present with a spectrum of clinicopathologic features as can noneoplastic myeloid infiltrates involving the skin such as neutrophilic dermatoses and infections. Distinction between reactive and neoplastic cutaneous myeloid infiltrates may occasionally be challenging. In this study, we used a commercially available Next-Generation Sequencing (NGS) Myeloid Kit to detect fusion genes and mutations in cases of leukemia cutis of myeloid lineage, and in nonneoplastic cases such as Sweet's syndrome, and evaluated its utility as an ancillary test in differentiating these disorders and its correlation with the original cytogenetic findings.

Design: Available slides (H&E & IHC) of 29 cases with the diagnosis of leukemia cutis or Sweet's syndrome were retrieved from our archives. Clinical history and slides were reviewed. Two cases of suppurative dermatitis were included as controls. FFPE blocks were selected and tumor RNA and DNA were extracted. Tumor RNA was subjected to the Archer™ FusionPlex™ Myeloid Panel and the results were analyzed using Archer ™ analysis software. Tumor DNA was subjected to Ion AmpliSeq™ Cancer Hotspot Panel v2 and analyzed using Variant Caller (v5.0.2.1). The Archer FusionPlex mutation and translocation results were compared to Ion Torrent hotspot and FISH results, respectively.

Results: 42% of the cases had high-quality RNA extracts. The Archer myeloid kit detected 75% of the expected fusions compared to FISH. It also found 1 novel previously unreported fusion (SRSF2-CIRBP) in a case of Leukemia Cutis and concurrent Acute Myeloid Leukemia with no other translocations identified by FISH. The myeloid kit was also able to detect pathogenic mutations in genes such as CEBPA, IDH2 and RUNX1, as well as many novel point mutations in all of the high-quality and 88% of low-quality specimens. Interestingly, a mutation in the KIT gene was detected in a case of Sweet's syndrome in a patient with concurrent AML.

Conclusions: The NGS myeloid panel proved to be an efficient tool in detecting both mutations and translocations in cutaneous myeloid infiltrates when there were enough cells to extract high quality RNA. The biggest challenge to widely using this panel in cutaneous myeloid infiltrates is to optimize the extraction of target cells that might be spread throughout the dermis. With more optimized microdissection techniques, NGS may become a reliable ancillary technique in differentiating neoplastic and reactive cutaneous myeloid infiltrates.

511 Delineation of Dermal Hyperneury as a New Entity. Report of Eight Cases

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Background: Dermal hyperneury is defined as the presence of increased number and size of myelinated and non myelinated nerve fibres in the dermis. It is a rare finding that is mainly known in the context of clinical syndromes such as classic and attenuated MEN2b, Cowden syndrome, neurofibromatosis, and in rare syndromic associations of medullary thyroid carcinoma. In these cases, it presents with multiple lesions in normal or lesional skin. Neuromas in MEN2b/Multiple mucosal neuromas have been defined as solitary haphazard bundles of Schwann cells or tortuous hyperplastic nerves with thick perineurium, which are not always well circumscribed. There are reports of multiple cutaneous or mucocutaneous neuromas in patients with negative syndromic screening. Localised, dermal hyperneury is encountered in areas of trauma/scarring, chronic rubbing/scratching, nodular prurigo, notalgia paresthetica and neurocristic hamartoma. Interestingly, PTEN and RET mutations involved in Cowden and MEN2b syndromes respectively, are implicated in common pathways of the growth and development of neural crest derived adn nerve tissue.

Design: We present eight cases spanning through the spectrum of conditions described. Four patients presented with multiple cutaneous papules, variably symptomatic.

Results: Extensive investigation including neurological and endocrine assessment did not reveal any syndromic stigmata for MEN2b, neurofibromatosis (type 2) or Cowden syndrome and there was no personal or family history of thyroid cancer. Immunohistochemistry revealed no PTEN loss, further in support of negative association with Cowden syndrome. Three cases were of localised forms: one case of notalgia paresthetica, one case of trauma, and one case of scarring. One patient presented with two cutaneous lesions with no other relevant findings.

Conclusions: We would like to propose dermal hyperneury as a distinct rare entity, specifically in presentations with multiple cutaneous lesions and no syndromic stigmata, and, therefore, no associated risk of malignancy.

512 Frequent Loss of T-Cell Receptor-Beta (TCRβ) Expression at Transformation in Mycosis Fungoides (MF/SS)

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Background: Mycosis fungoides (MF) arises from mature T-cells with a CD4+ phenotype and TCRβ derivation. In rare instances cases with CD8+ and/or TCRγ phenotype may be observed posing diagnostic confusion with other T-cell lymphomas. Given the aggressive nature of CTCL with TCRγ phenotype, we sought to investigate the phenotype of large cell transformed-MF (T-MF) to 1] examine the TCR signature of the transformed component 2] to assess the frequency of phenotype switch (TCRβ to TCRγ) and occurrence of "TCR silent" (TCRβ-/ TCRγ -) populations.

Design: A total of 8 formalin-fixed skin biopsy tissues comprising 6 T-MF and 2 transformed Sezary syndrome (T-SS) were immunostained using the following antibodies: CD3, CD4, CD8, CD20, CD30, TCRβ, and TCRγ, TCRβ and TCRγ. Expression was examined in the transformed large cells and the background small neoplastic T-cells. Additionally, one case of MF with dual TCRβ and TCRγ was examined in conjunction for comparison.

Results: All eight biopsies showed TCRβ+/ TCRγ- signature in the small neoplastic T-cells. On examination of the large cells, 4 of 8 biopsies showed large cells that were variably double negative for both TCRβ and TCRγ ("TCR-silent"). These were ascertained to be CD3+/CD20- cells confirming T-cell derivation. One of these 4 showed $CD3^{dim}$ phenotype, while another showed loss of CD4. Large cells in the remaining four biopsies were uniformly TCRβ+/ TCRγ-, concordant with the untransformed component without any obvious association with high stage.

Conclusions: A significant subset of large cells in transformed MF/SS lose TCRβ at transformation without evidence of any TCR switch, although the prognostic significance of this finding is unclear. Longer follow up in a larger cohort will clarify the significance of these findings.

513 Targeted DNA and RNA Sequencing in Malignant Peripheral Nerve Sheath Tumors (MPNST) AND Spindle AND Desmoplastic Melanomas (SDM): Novel Findings

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Background: MPNST is a rare soft tissue neoplasm sharing morphological features and some molecular events with SDM. Herein, we investigate molecular events that may serve as potential targets for clinical trialsas and biomarker for prognosis in both entities.

Design: DNA and RNA from formalin-fixed paraffin-embedded (FFPE) tissue were extracted. Massive high-throughput deep parallel sequencing was performed using oncomine comprehensive panel detecting relevant SNVs, CNVs, gene fusions, and indels from 143 unique genes for clinical trial research programs. In silico prediction models were used to assess the effect of events. Log rank and Pearson test were used .

Results: 51 patients (SDM/DM=16/11, MPNST=24, male n[thinsp]=[thinsp]37, female n[thinsp]=[thinsp]16) had sufficient material for testing. The average age was 56 years (33-80) and median follow up was 16 months (2-120 months). Activating *NOTCH1* mutations affecting the *EGFR* like domain, and *KDR* mutations were identified in 60% of SDM and 35% MPNST. *NF1* deleterious mutations were identified in 73% of MPNST and 67% of SDM . Inactivating mutations of *TSC1/TSC2* were identified in 35% of MPNST and 40% of SDM. *PTCH1* events were detected in 40 % of SDM and 31 % of MPNST. Other deleterious events involving tumor suppressor genes including *CDKN2A* (47% SDM, 27%MPNST), *TP53* (60% SDM, MPNST 38%) and *RB1* (28% SDM, 4% MPNST) were also detected. There was a moderate positive correlation between *PTCH1* events and Breslow Thickness in the SDM group (R=0.5359, p=0.02). No significant correlation was identified between any of these events and DFS or OS. RNA sequencing was negative for fusions involving known oncogenes. Copy number analyses showed no aberrations in all SDM cases. Partial trisomies in chromosome 5, 7 and 8 were seen in 33%, 41% and 25% of MPNST cases. Isolated locus CN gains in *FGFR4, MYC,* and *EGFR* were detected in 20% of the MPNST cases.

Conclusions: Our results suggest that angiogenesis genes (*KDR* and *NOTCH1*) and mTORC pathway represent promising targets in MPNST and SDM for ongoing clinical trials.t Testing using a cancer immunological panel targeting genes that affect response to immunotherapies is underway.

514 In-Situ Protein Expression of Melanocyte Differentiation Antigen Tyrosinase-Related Protein-1 (TRP1)

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Background: Melanocyte Differentiation Antigens (MDAs) are expressed in cells and tumors of melanocytic lineage. MDAs such as gp100, Melan-A/MART1, and tyrosinase and their corresponding antibodies HMB45, A103, and T311 serve as important diagnostic tools in surgical pathology. MDAs are also employed as vaccine targets for the immunotherapy of melanoma. However, little is known about the expression of TRP1, another MDA which acts as an enzyme in melanin biosynthesis.

In the present study, we tested a novel anti-TRP1 monoclonal antibody (mAb), clone EPR13063, as a potential reagent in surgical pathology and analyzed the expression of TRP1 in panels of normal and tumor tissue.

Design: MAb EPR13063 (Abcam; ab178676)) was obtained commercially. Immunohistochemcial (IHC) staining was performed on a Leica-Bond-3 IHC stainer platform. EPR13063 was tested on formalin-fixed paraffin-embedded archival tissues, incldluing normal tissues, non-melanocytic and melanocytic lesions such as nevi, primary and metastatic melanomas, as well as PEComas.

Results: EPR13063 worked well in standard archival tissue. In normal tissue, there was consistent labelling of melanocytes. No immunostaining was observed in other normal tissues, including all parenchymal organs. 10/10 compound nevi were TRP1-positive with most intense staining at the dermoepidermal junction; staining intensity decreased with maturation of the nevocytes towards the lower dermis. 15/15 primary melanomas were TPR1-positive, and 10/15 displayed homogeneous expression with >50% of the tumor cells immunopositive; one case was immunopositive in <5% of the tumor. In metastatic melanoma, 7/20 tumors were TRP1-positive displaying a mostly homogeneous expression. 5 desmoplastic melanomas were negative. 9/11 PEComas were heterogeneously TRP1-positive. 35 primary tumors from breast, colon, kidney, ovary, testis, lung and liver were all negative.

Conclusions: TRP1 belongs to the family of MDAs such as Melan-A, TRP2, tryosinase and gp100. Although all MDAs play important roles in the biology of melanoma, little is known about the expression of TRP1. Here we show that commercial mAb EPR13063 is a useful reagent for the detection of TRP1 in surgical pathology. TRP1 shows a consistent presence in normal melanocytes as well as nevocytes. In primary melanomas, TRP1 is homogeneously expressed in most tumors of our series and compares to other MDAs such as Melan-A or gp100 (HMB45 antigen) but shows a lower expression in metastatic melanoma. As with other MDAs, TRP1 is present in PEComas but negative in desmoplastic melanoma.

515 Factors Affecting Accuracy of Clinical Diagnosis of Suspected Cutaneous Melanomas, a 5-Year Retrospective Study of 17,669 Melanomas

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Background: Skin cancer is on the rise, with over 70,000 new melanomas diagnosed each year in the US, highlighting the need for clinicians to accurately recognize and biopsy suspicious lesions. Previous studies investigated the accuracy of clinicians' identification of melanoma prior to biopsy, but few examined what factors impact this accuracy. We examine clinician accuracy for melanoma diagnosis and assess factors that correlate with improved diagnostic accuracy.

Design: We examined data from all cutaneous wet tissue biopsies at a tertiary academic care center (N=35,680) and all wet tissue biopsies sent by external groups to the same institution's dermatopathology lab for processing and diagnosis (N=492,398) for the years 2011-2015. We defined clinically suspicious lesions as biopsies with the clinical text "melanoma," "MM," or "MIS". Accuracy was defined as the percent of clinically suspicious lesions that were histologically confirmed to be invasive or in situ malignant melanoma, or positive predictive value (PPV). We examined the variables that were associated with higher accuracy and higher PPV using multivariate logistic regression.

Results: 53,931 biopsies were clinically suspicious for melanoma (10%). Of these lesions, dermatopathology confirmed 10,104 melanomas (PPV=19%). Stratified analysis revealed an academic clinician PPV of 25% in 5,390 suspicious biopsies and external clinician PPV of 18% in 48,541 suspicious biopsies.
Factors that predicted a higher clinician PPV included patient's male gender, words 'pigmented' and 'invasive' in the clinical text, and locations on the face, arm and foot. The terms 'nevus' and 'atypia' in the clinical text and biopsy location on the hand or scalp were associated with lower PPV. Diagnoses noting 'margins free' were associated with increased PPV, while 'margin involved' was associated with lower PPV. A fragmented specimen was associated with lower PPV.
Conclusions: The overall clinical diagnostic accuracy of malignant melanoma was 19%. A clinician's free text on the biopsy requisition sheet and fragmented nature of submitted wet tissue biopsies may affect clinical diagnostic accuracy and correct histologic classification.

516 To Evaluate the Utility of PAS Stained Skin Scrape Cytology Smear in Clinically Suspected Fungal Infections of the Skin

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Background: Fungal skin infections form a significant burden in routine dermatology practice. For fungal dermatitis, a skin scrape and a wet KOH preparation form the routine practice for diagnosis.
Design: This prospective study was carried out in a span of 4 months on the patients clinically diagnosed with fungal dermatitis and fungal infections of the skin. For each patient a wet KOH preparation and one PAS stained skin scrape cytology(SSC) smear was prepared. Tinea capitis and tinea pedis were excluded as crushing of scrapings in such cases would be difficult.
Results: Out of the total 52 suspected cases of fungal infections of skin, 50 showed fungal elements on either or both the techniques. These 50 patients aged in range from 1 year to 62 years with a mean age of 28.8 years and maximum number of patients falling in the age group of 11-30years (66%). Male to female ratio was 2.57:1.The presentation was Tinea cruris together with Tinea corporis in 21 cases(42%), isolated tinea cruris in 19cases(38%), tinea corporis in 8 cases(16%), tinea corporis with tinea manuum with onychomycosis in 2 cases(4%). Out of the 50 patients 12(24%) had not received any treatment. Co-morbid conditions were seen in 6 (12%) patients out of which diabetes was the most common. KOH preparation was positive in 45 out of 50 cases (90%) and skin scrape cytology was positive in 49 out of 50 cases (98%). The sensitivity,specificity, positive predictive value and negative predictive value for PAS stained skin scrape cytology smear was 98%,100%,100%,66% and that for KOH preparation was 90%,100%,100%,28% respectively.
Fungal elements quantity was graded on SSC smear as 1+(5), 2+(19),3+(19),4+(6)and in one case it was negative. The five cases in which KOH was found to be negative were having low grade positivity (two cases having 2+ positivity and 3 cases having 1+ positivity). Out of the total 49 cases that were positive on PAS, 45 cases showed hyphae only or hyphae more than yeasts, 2 cases showed yeasts more than hyphae and two cases showed 'spaghetti and meat balls' appearance. The average turnaround time taken for KOH preparation was 20 min and that of PAS stained skin scrape cytology smear preparation was 1hour 40 minutes.
Conclusions: : Incorporation of the PAS stained SSC smear for fungal dermatitis ensures more efficient and confident diagnosis. The slides can be filed and are available for archival studies unlike KOH preparation which has to be viewed promptly and discarded thereafter.

517 The Incidence of Radiotherapy-Induced Angiosarcoma in the Skin After Treatment for Breast Cancer in Denmark. A Population-Based Study

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Background: Angiosarcomas (AS) of the skin are seen in three clinical settings: 1) UV-induced AS after long-term sun exposure, 2) in chronic lymphedema and 3) following radiotherapy (RT). Adjuvant RT after breast-conserving surgery has increased through recent years, but the exact incidence of RT-induced AS in women treated for breast cancer is unknown.
Design: Data from all women diagnosed and treated for breast cancer in the period 1995-2015 were identified in the national database of the Danish Breast Cancer Cooperative Group (DBCG). In the same time period, AS cases in the breast region were identified by searching the Danish National Pathology Database.
Results: Forty-one cases of AS in the breast were identified, of which 38 (92.6%) occurred in patients with at least 10-years follow-up. This allows us to calculate the incidence of RT-induced AS in the first 12 years of the study period with 92.6% probability. As AS in the vast majority of cases presents within 10-years after RT, the incidence of RT-induced AS was found to be 0.27%. In addition, 7 other types of sarcomas were identified in the same population giving a total incidence of RT-induced sarcomas of 0.32%.
Conclusions: We present the exact incidence of sarcomas in women treated with RT for breast cancer over a period of 12 years with at least 10-years follow-up.

518 Diagnostic Distinction of Malignant Melanoma and Benign Nevi by a Gene Expression Signature and Correlation to Clinical Outcomes

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Background: Biomarker gene expression quantification shows utility as a diagnostic adjunct for ambiguous melanocytic lesions. A clinically validated 23-gene signature showed greater than 90% diagnostic accuracy compared to expert consensus histopathologic diagnosis. Here the sensitivity and specificity of this molecular test was additionally tested against clinical outcome based classification of melanocytic lesions.
Design: Expression of 23 genes was measured (qRT-PCR from FFPE biopsies) from 99 primary melanomas that ultimately metastasized and 83 benign nevi. A weighted algorithm was applied to the expression levels to produce a single numeric score, and the sensitivity and specificity of the score was determined based on clinical outcome-proven diagnosis.
Results: Overall, the gene expression score differentiated malignant melanoma from benign nevi with a sensitivity of 93.8% and a specificity of 96.2%. In outcome-proven superficial spreading melanoma, the score classified 29/31 cases as malignant, 1 as benign, and 1 as indeterminate. Of the nevi presenting with dysplastic features, 10/14 were classified as benign, 3 as malignant, and 1 as indeterminate.
Conclusions: The gene expression score distinguishes melanoma from nevi with a high degree of accuracy as compared to the diagnostic standard of clinical outcome. It is possible that some of the apparent false positives observed in dysplastic nevi may have been melanomas that were 'cured' by the initial biopsy or excision. In such cases, the gene expression signature can provide additive information to support excision and close clinical surveillance.

519 Primary Cutaneous CIC-DUX Sarcoma: A Report of Two Cases

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Background: CIC-DUX sarcomas are part of a recently described subset of round cell sarcomas with significant morphologic, immunophenotypic and clinical overlap with Ewing sarcoma. CIC-DUX sarcomas are defined by recurrent translocations of *CIC* (chromosome 19) fused with either *DUX4* (chromosome 4) or its paralog *DUXL4* (chromosome 10). They are known to occur primarily in the deep soft tissue of the trunk and extremities of young adults, and to exhibit aggressive biologic behavior.
Design: We report two cases of primary cutaneous CIC-DUX sarcoma received in consultation. The Archer® FusionPlex® Sarcoma Kit targeted sequence assay was utilized to probe for fusions of 26 known sarcoma-associated genes (RT-PCR confirmed). Other testing included interphase FISH for rearrangements involving the *EWSR1*, *FUS*, and *CAMTA1* genes and immunohistochemistry including CD99, CD31, ERG, and INI-1.
Results: Cases 1 and 2 presented as ulcerated nodules on the forearm of a 65-year-old man and thumb of a 55 year-old woman, respectively. The tumors were composed of uniform small to medium sized cells with round to oval nuclei with vesicular chromatin, variably prominent nucleoli and pale eosinophilic to clear cytoplasm, arranged in multiple geographic tumor nodules and sheets in a hyalinized stroma. Scattered mitotic figures were present. Case 1 had focal pagetoid involvement of the overlying epidermis. Both cases were positive for CD99 (strong, membranous), focally positive for CD31, and showed patchy nuclear staining for ERG. INI-1 was retained and all other stains were negative. FISH testing for rearrangements of *EWSR1*, *FUS* and *CAMTA1* were negative. Archer® FusionPlex® Sarcoma Kit revealed a positive t(4;19)(q35;q13) *CIC-DUX4* translocation in both cases. In case 1, the patient was treated with wide excision followed by radiation and is ANED 9 months later. In case 2, the patient underwent hand amputation then developed two left lower lung nodules (1.5cm and 0.7cm) 15 months later, which were excised. Now at 26 months out, she is alive but progressing with multiple metastases (lungs, mediastinum and soft tissue).
Conclusions: CIC-DUX sarcomas can show primary presentation in the skin, and should be diagnostically considered in any case exhibiting histologic and immunophenotypic features reminiscent of Ewing sarcoma. In contrast to most cutaneous Ewing sarcoma, cutaneous CIC-DUX sarcomas may behave in an aggressive fashion, though additional studies are needed to confirm this finding. PCR is useful in discriminating this entity.

520 Genetic Alteraions in Lesions of Anogenital Mammary-Like Glands and Their Mammary Counterparts

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Background: Lesions affecting anogenital mammary–like glands (AGMLG) are histopathologically very similar to those seen in the breast. The information on molecular biological mechanism in these tumors is scarce. One can assume that the striking morphological similarity of tumors of AGMLG to their mammary counterparts may be also reflected at the genetic level.
Design: We analyzed the mutational profile of 16 tumors of AGMLG (hidradenoma papiliferum (HP) and fibroepithelial neoplasms) and its 18 breast counterparts by high-coverage next generation sequencing (NGS) using a gene panel comprising 50 cancer-related genes and, additionally, for mutations in the *MED12* gene were studied. All mutations detected by NGS were independently validated by Sanger sequencing (concordance: 100%).

Results: The main clinicopathological features of the cohort and detected mutations are summarized in Table 1.

	HP	Breast intra-ductal papil-loma	Anogenital fibroad-enoma (FA)	Breast FA	Ano-genital Phyllodes tumor (PhT)	Breast PhT
Number of cases	5	6	8	9	3	3
Age (years) Mean (range)	35.2 (25-45)	57.7 (44-75)	49.9 (30-70)	34.7 (22-53)	37.7 (31-44)	47 (42-51)
Size (cm) Mean (range)	0.9 (0.6-1.2)	0.9 (0.5-1.5)	2 (0.5-3)	1.9 (1.1-3.5)	2.9 (2-4)	1.7 (1.2-2.5)
Location-vulva-perineum-perianal	5 (100%)		6 (75%) 1 (12.5%) 1(12.5%)		2 (66.7%) 1 (33.3%)	-
Number of cases with mutation in genes (except MED12)	1 (20%)	3 (50%)	2 (25%)	-	1 (33%)	2 (66.7%)
Gene/ protein	PIK3CA/ p.Glu545 Lys	AKT1/ p.Glu17 Lys	AKT1/ p.Glu17 LysMET/ p.Arg988 Cys	-	ABL1/ p.Lys266 ArgTP53/ p.Arg110 Cys	SMAD4/ p.Gln256 LeuKIT/ p.Asp816 AsnFBXW7/ p.Thr482 AlaRET/ p.Ser767 Asn
No mutation	3	1	1	3	1	1
Not analyz-able	1	2	-	2	1	-
Not per-formed	-	-	5	4	-	-
Number of mutations in MED12 (protein)	-	-	-	3 (33%) (p.G44D p.Gly44Cys p.Gly44Ser)	-	-

Conclusions: We demonstrated various genetic alterations in lesions arising from AGMLG, including several novel mutations. Our study expands the spectrum of lesions of AGMLG harboring mutations in genes encoding the PI3K-AKT cascade. *AKT1* or *PIK3CA* mutations of PI3K-AKT cascade play a role both in tumors of AGMLG and their mammary counterparts. Some histopathologically similar anogenital and breast lesions appear to share similar molecular pathways.

521 Spectrum of changes in Anogenital Mammary-Like Glands (AGMLG) in Primary Extramammary Paget Disease (EMPD)

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Background: Primary EMPD is a rare neoplasm with uncertain histogenesis usually involving the anogenital area.

Design: To determine if a subset of primary EMPD may originate in AGMLG, we studied 180 specimens (101 from wide surgical excisions (median: 10 blocks per case) and 79 small specimens (1 block per case)) from 149 patients with EMPD. The following features were assessed, using the same criteria applied in breast pathology: oxyphilic metaplasia (OM), columnar cell change (CCC), columnar cell hyperplasia (CCH), usual ductal hyperplasia (UDH), atypical ductal hyperplasia (ADH), ductal carcinoma in situ (DCIS), invasive mammary-type carcinoma and colonization by Paget cells.

Results: Alterations in AGMLG were identified in 33 specimens from 31 patients with EMPD involving vulva or perianal area and are summarized in Table.

Type of changes in AGMLG	Number of cases	Other changes in AGMLG (number of cases)	Age (years) Median (range)
CCC	12	-	68 (49-86)
CCH	2	CCC(2)	61 (57-65)
UDH	5	CCC(4), OM (1)	70 (59-93)
DCIS, G2-3	3	CCC(3), AGMLG colonization (1)	51 (40-56)
Invasive ductal carcinoma (IDC), G3, DCIS	1	-	74
Invasive mammary-type carcinomas, G2-3	3	IDC(2), tubulo-lobular carcinoma(1), CCC(3), CCH(1)	78 (38-78)
Colonization by neoplastic cells	5	CCC(3), UDH(2), ADH(1),OM(1)	51.5 (47-75)

Conclusions: AGMLG in primary EMPD manifest a range of changes analogous to those occurring in the breast and which may be regarded as a morphologic spectrum. Since CCC and CCH may be encountered in normal AGMLG, these alterations would seem to play little, if any, role in the pathogenesis of EMPD. However, DCIS and invasive carcinoma comprising the malignant end of the spectrum suggest, by analogy with some cases of mammary disease that rare cases of primary EMPD may originate in AGMLG with subsequent upward migration of the neoplastic cells into the epidermis and possible later breach through the basement membrane. UDH and ADH can then be regarded as precursor lesions, linking both ends of the spectrum. If our proposition is confirmed in subsequent studies, this may result in re-evaluation of current therapeutic approaches to primary EMPD, especially with respect to local surgical and nonsurgical treatment.

522 Basal Cell Carcinoma with Matrical Differentiation: Clinicopathological, Immunohistochemical and Molecular Biological Study of 22 Cases

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Background: Basal cell carcinoma (BCC) with matrical differentiation, with about 30 cases documented mainly as isolated cases report. We studied a large series of this neoplasm, including cases with an atypical matrical component, a hitherto unreported feature.

Design: Lesions coded as BCC with matrical differentiation were reviewed; 22 cases were included. Immunohistochemical (IHC) studies were performed using antibodies against BerEp4, β-catenin, and EMA. Molecular genetic studies using Ion AmpliSeq Cancer Hotspot Panel v2 by massively parallel sequencing on Ion Torrent PGM were performed in 2 cases with an atypical matrical component (one was prior subjected to microdissection to sample the matrical and BCC areas separately).

Results: There were 13 male and 9 female patients, ranging in age from 41 to 89 years. Microscopically, all lesions manifested at least two components, a BCC area (follicular germinative differentiation) and areas with matrical differentiation. A BCC component dominated in 14 cases, whereas a matrical component dominated in 4 cases. Matrical differentiation was recognized as matrical/supramatrical cells (n=21), shadow cells (n=21), bright red trichohyaline granules (n=18), and blue-grey corneocytes (n=18). In 2 cases matrical areas manifested cytological atypia, and a third case exhibited an infiltrative growth pattern, with the tumor metastasizing to a lymph node. BerEP4 labelled follicular germinative cells, whereas it was markedly reduced or negative in matrical areas. The reverse pattern was seen with β-catenin. EMA was negative in BCC areas but stained a proportion of matrical/supramatrical cells. Genetic studies revealed mutations of the following genes: *CTNNB,1 KIT, CDKN2A, TP53, SMAD4, ERBB4* and *PTCH1,* with some differences between the matrical and BCC components.

Conclusions: Matrical differentiation in BCC in most cases occur as multiple foci. Rare neoplasms manifest atypia in the matrical areas. ICH for BerEP4, EMA and β-catenin can be helpful in limited biopsy specimens. From a molecular biological prospective, BCC and matrical components appear to share some of the gene mutation spectrum but have differences in others, but this observation must be validated in a large series.

523 Isocitrate Dehydrogenase 1 (*IDH1*) Mutations in Melanoma Frequently Co-Occur with *NRAS* Mutations

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Background: Isocitrate dehydrogenase 1 (IDH1) is a metabolic enzyme that converts isocitrate to α-ketoglutarate. *IDH1* mutations are associated with the accumulation of the oncometabolite D-2-hydroxyglutarate, which acts as an epigenetic modifier, and the development of multiple malignancies including acute myeloid leukemia and certain gliomas. Here, we examine the features of melanomas with *IDH1* mutations.

Design: From May 2013 – July 2016, 186 melanoma samples from patients with regional lymph node involvement or distant metastatic disease were tested for somatic mutations with the 50 gene AmpliSeq Cancer Hotspot Panel v2. DNA was extracted from unstained FFPE slides with a minimum tumor cellularity of 10% and was quantified using the PicoGreen method. Barcoded libraries were prepared using 10ng of DNA and sequencing performed on the Ion Torrent PGM (318 chip). Variants were identified using the Ion Torrent Variant Caller Plugin and reference genome hg19. Golden Helix's SVS software was used for annotation and prediction of the significance of the variants.

Results: One hundred sixty-nine samples were successfully sequenced. Sequencing results divided the majority of cases into three main groups: *BRAF* mutant (74/169; 44%); *NRAS* mutant (33/169; 19.5%) and those that lacked a driver mutation/wild type (WT) (of note, *NF1* alterations are not detected by the AmpliSeq assay). Seven melanomas (4%) were positive for *IDH1* p.R132C. In six cases, the tumors also had a co-existing *NRAS* mutation (p.Q61X); the seventh was WT. Two cases had a *TP53* mutation and one case an *ATM* variant. The patients' age ranged from 59-78 years and 2/7 patients were female. Three patients were stage 3; four stage 4. One is deceased, two alive with stable disease on pembrolizumab, two have no evidence of disease and two are lost to follow up.

Conclusions: *IDH1* mutations are identified in a subset of melanomas and, in our population, are significantly associated with co-existing *NRAS* mutations ($p = 0.0002$). In the TCGA data (N = 287; cbioportal, accessed 9-24-26) *IDH1* p.R132X mutations co-occur with both *NRAS* (6/12) and BRAF (3/12) mutations and are highly associated with the CIMP (CpG island methylator phenotype). *IDH* mutations may define a unique subset of melanoma patients who are eligible for IDH1 targeted therapies or combined therapies, such as MEK inhibitors when there is co-existing *NRAS* mutations, or immunotherapy.

524 SF3B1 R625 Mutations Occur in Non-Uveal Melanomas at a High Frequency

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Background: Splicing factor 3B subunit 1 (*SF3B1*) SF3B1 is a component of the U2 small nuclear ribonucleoprotein complex (snRNP) that participates in the splicing of pre-mRNAs, and a component of the minor U12-type spliceosome. Recurrent mutations in exons 12 and 15 of SF3B1 were initially demonstrated in myelodysplastic syndrome and chronic lymphocytic leukemia. More recently, recurrent somatic SF3B1 mutations at residue R625 have been reported in 19% of uveal melanoma (Harbour *et al.* 2013), and are associated with an improved clinical course versus BAP1-mutated uveal melanoma. It has been suggested that SF3B1 R625 mutations may be a genetic alteration specific to uveal melanoma along with mutations in GNAQ, GNA11, and EIF1AX. In this study, we examined the distribution of SF3B1 variants in various melanoma histologic subtypes.
Design: Somatic mutation data was gathered from targeted next generation sequencing (309 genes) of all histologic melanoma subtypes (n=163), including SF3B1-mutated melanomas from 14 patients at our institution between 2013-2016. Limited clinical and surgical pathological data was abstracted from our next-generation sequencing database.
Results: SF3B1 mutations occurred in 8.6% of all melanomas (14/163), of which 86% (12/14) occurred at position R625. 75% (9/12) of melanomas harboring SF3B1 R265 mutations were of mucosal origin, with the remainder being of ocular origin. SF3B1 R625 mutations in mucosal melanoma were mutually exclusive of GNAQ mutations (0/9), whereas SF3B1 R625-mutated ocular melanomas were significantly enriched for co-occuring GNAQ mutations (3/3; p=0.0045). In addition, SF3B1 R625-mutated melanomas had a significantly lower non-synonymous mutation burden than SF3B1 wildtype melanomas (p = 0.001).
Conclusions: The majority of SF3B1 R625 mutations in melanomas at our institution in this limited study set occur in mucosal melanoma. Additionally, SF3B1 mutations in mucosal melanoma appear to be mutually exclusive from GNAQ mutations, which tend to co-occur with SF3B1 mutations in ocular melanomas, perhaps suggesting a unique biology in mucosal melanomas. Consistent with their predominantly non-cutaneous origin, SF3B1 R625-mutated melanomas have a lower non-synonymous mutation burden than SF3B1 R625 wildtype melanomas.

525 Expression of PD-L1 in Extramammary Paget Disease: Implications for Immune-Targeted Therapy

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Background: Extramammary Paget disease (EMPD) is a rare adenocarcinoma seen mostly in elderly individuals with frequent genital or perianal location. It has a high rate of local recurrence with rare nodal and systemic metastasis. Due to the paucity of clinical studies examining therapeutic options for EMPD, there is no universally accepted standard of care. Interaction between Programmed Death-1 (PD-1) receptor on cytotoxic T-cells and its ligands on other immune and tumor cells is a potential mechanism by which neoplastic cells evade tumor immunity. Immune checkpoint inhibitors have been recently approved by FDA for treatment of melanoma, etc.
Design: PD-L1 expression was assessed by immunohistochemistry using an FDA-approved mouse monoclonal antibody (clone 22C3; Dako) on 21 EMPD and 10 mammary Paget disease (MPD) cases. A 1% cutoff value for positive staining was used based on FDA-approved criteria. Evaluation of PD-L1 expression was performed independently by two pathologists and scored by multiplying staining intensity by percentage of positive tumor and immune cells (H-score).
Results: The mean age of EMPD was 67.7 years with no sex predilection. 4/21 cases had associated invasive carcinoma. 3/21 patients (pts) died of disease with a mean follow-up of 29.8 (range: 10.3 – 46.2) months. 7/21 pts had local recurrence. 8/21 EMPD and 1/10 MPD cases had positive PD-L1 staining within tumor cells and 17/21 EMPD and 10/10 MPD cases had positivity in the associated lymphocytic infiltrate. With the exception of one EMPD case, which showed nuclear positivity, all other cases showed cytoplasmic and/or membranous positivity. There was a significant correlation of PD-L1 expression in lymphocytes with perianal EMPD and HER2/neu expression in MPD pts (p=0.03 both). There was no other significant difference in PD-L1 positivity (H-score) and clinical parameters such as age, sex, metastasis, local recurrence, overall survival and disease specific survival, which may be due to relatively low sample size of this rare disease.
Conclusions: In our series, PD-L1 is positive in 84% (26/31) of Paget disease within tumor cells or associated immune infiltrate. To our knowledge, this is the first study evaluating PD-L1 expression in Paget disease. These results raise the possibility of effectiveness of inhibition of PD-1/PD-L1 axis in EMPD and MPD pts.

526 Expression of Markers for Pericytes and Myofibroblasts in Bleomycin (Bleo)-Induced Dermal Fibrosis: Potential Role of Neuropeptide Receptors in a Mouse Model for Scleroderma (SSc)

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Background: SSc is a chronic collagen-vascular disease that manifests initially with dermal fibrosis, then later progresses to multiple organ fibrosis. There is no treatment to arrest SSc. Recently, a mouse model of SSc was reported, in which Bleo is injected intradermally (ID) for 21-28 days. Two labs showed that reactive oxygen species (ROS) cause this dermal fibrosis. We previously observed that ROS trigger GRP-mediated pulmonary fibrosis to hyperoxia or radiation. We first verified that ROS trigger this dermal fibrosis, and now test the hypothesis that gastrin-releasing peptide (GRP) from cutaneous nerves has a role in this process by acting on myofibroblasts [alpha-smooth muscle actin, SMA+] and pericytes [SMA+ and neural/glial antigen 2, NG2+]. We tested expression of both GRP receptors, GRPR and neuromedin B receptor (NMBR) by immunohistochemistry.
Design: Flanks of 10-wk old C3H/HeJ females were injected ID with Bleo (100-μg) 5d/wk for 3-wks. Some mice also received antioxidant N-acetylcysteine (NAC) IP, and other Bleo mice received GRP blocking mAb-2A11. After 21d, lesions paraffin sections were immunostained for SMA, NG2, GRPR, or NMBR. Relative extent of immunostaining in dermis and epidermis was scored by 2 observers (EK & MES) on a scale from 0-3, comparing prevalence of (+) cells (0, 1=detected in few cells, 2=many cells (+), & 3=most cells positive.
Results: Bleo induced >10-fold increased pericytes & myofibroblasts, in dermis ($P<0.001$), and NG2 and SMA staining scores were linearly correlated ($R^2 = .87$, $P<0.05$). SMA & NG2 were reduced by NAC (~80% decrease, $P<0.001$) or mAb2A11 (~50% decrease, $P<0.01$), similar to our prior studies of dermal thickness. Epidermal scores for GRPR were significantly decreased in Bleo+2A11 mice compared to Bleo alone (0.5 ± 0.3, 1.9 ± 0.3, $P < 0.005$), like prior studies of GRPR up-regulation by GRP. However, there were no other differences in GRPR between study groups. NMBR scores were unchanged amongst the groups.
Conclusions: In the mouse model of scleroderma, increased pericytes and myofibroblasts occur in regions of dermal fibrosis. Although GRPR &/or NMBR could contribute to Bleo-induced dermal fibrosis their expression is unchanged between groups. We previously determined that GRP induces GRPR gene expression. Regardless, sustained epidermal expression of both receptors would be consistent with potential GRP signaling in epidermis as a mechanism for epidermal hyperplasia and dermal fibrosis, such as through epithelial-mesenchymal transformation.

527 Nuclear BRAF^{V600E}: Its Clinical Significance as a Prognostic Marker for Melanoma Aggressiveness

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Background: Approximately 50% of cutaneous melanomas harbor a *BRAF* gene mutation, resulting in constitutive activation of the mitogen-activated protein kinase (MAPK) pathway. The advent of small molecule FDA-approved inhibitors of BRAF and MEK has revolutionized management and improved progression-free survival in metastatic melanoma. Accordingly, *BRAF*^{V600E} status is now of clinical importance. Immunohistochemistry (IHC) using monoclonal VE1 antibody specific to the V600E mutation is a highly reliable method for detection of BRAF^{V600E} and may be appropriate as an initial test in place of DNA-based assays. We studied whether site-specific intracellular localization of BRAF^{V600E} has clinical significance in primary melanoma.
Design: IHC analysis of BRAF^{V600E} was performed on formalin-fixed paraffin-embedded specimens of primary melanoma (n = 84). Staining intensity was graded on a scale of 0 to 3 in a blinded manner. Localization was recorded as cytoplasmic and/or nuclear. Correlation to clinical factors was analyzed by fisher's exact test and two-tailed t tests.
Results: We included 84 patients, of which 74% were male and with mean age of 64 years. 39 cases were classified as stage 1, 42 cases as stage 2 and 3 cases as stage 3. The frequency of *BRAF*^{V600E} mutation by immunohistochemistry was 54% (45/84). Cytoplasmic BRAF^{V600E} expression was positive in 35% (29/84). Nuclear BRAF^{V600E} expression was positive in 29% (24/84). Overall BRAF^{V600E} expression correlated with age (p = 0.009), location (p = 0.035), clinical stage (p = 0.024), mitotic activity (p = 0.010) and ulceration (p = 0.045). Cytoplasmic BRAF^{V600E} expression correlated with age (p = 0.007) and gender (p = 0.035). Nuclear BRAF^{V600E} expression correlated with clinical stage (p = 0.001), tumor stage (p = 0.001), nodal stage (p = 0.021), invasion (p = 0.002), Clark level (p = 0.001), mitotic activity (p = 0.001), ulceration (p = 0.001), cytology (p = 0.029) and margin status (p = 0.007).
Conclusions: Our data suggests that nuclear localization of BRAF^{V600E} is associated with melanoma aggressiveness. Further study is needed to confirm the clinical relevance of nuclear location of BRAF^{V600E} as a prognostic and/or predictor marker for melanoma treatment.

528 BRAF V600E Mutation and P16 Expression of Melanoma in Nigerians: A 10 Year Retrospective Study

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Background: Melanoma (MM) is the most lethal of all skin malignancies worldwide. In blacks, it is associated with greater morbidity and mortality compared to Caucasians. Studies have shown that MM with BRAF V600E mutation and those with loss of p16 protein expression are associated with aggressive behaviour and worse prognosis. BRAF kinase inhibitors have been approved for treatment of BRAF V600E mutant cases.
Design: The aim of the study was to determine the BRAF V600E mutation protein expression and loss of p16 expression of MM cases in Lagos Nigeria within the last 10 years and to correlate these with prognostic markers. FFPE tissue blocks and corresponding archival H&E stained slides of all confirmed MM cases from January 2005 to December 2014 were retrieved. Immunohistochemical (IHC) analysis was carried out to determine mutant BRAF V600E and p16 protein expression using the VE1 and anti-CDKN2A/p16INK4a monoclonal antibodies respectively.
Results: During the study period, 52 MM cases were histologically diagnosed constituting 1.0% of total solid malignancies; 43 of these were cutaneous accounting for 19.7% of all skin malignancies and the 3rd commonest skin malignancy. The peak age was the 5th decade and male to female ratio was 1: 1.5. Cutaneous, mucosal, metastatic and ocular MM constituted 83%, 7%, 6%, and 4% respectively while 58% of the cases

occurred on the foot. The cutaneous cases were Nodular (84%), Acral lentiginous(14%) and Desmoplastic(2%) MM; 88% were in Clark's stage IV and V, 84% had Breslow thickness ≥4mm and ulceration was present in 60% of the cases.
IHC was carried out on 45 cases which consisted of 35 cutaneous, 3 musosal, 2 ocular and 3 metastatic (lymph nodes) MM. BRAF V600E mutation was detected in 12% while there was loss of p16 expression in 69% of the cases. There was however no significant correlation between these mutations and prognostic factors such as patients' age, histologic variants, Clark's level, Breslow thickness and ulceration as p-value in each case was >0.05.
Conclusions: Few MM cases in this study were BRAF V600E mutant, suggesting that most are BRAF *wt* and as such BRAF kinase inhibitors will only be effective in treating very few of our patients. However, majority had loss of p16 protein expression which in combination with delayed presentation may be responsible for the advance and aggressive tumours seen in our environment.

529 MET Gene High Copy Number (Amplification/Polysomy) Identified in Melanoma for Potential Targeted Therapy

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Background: Mesenchymal epithelial transition factor (MET) gene, a proto-oncogene, encodes a receptor tyrosine kinase, and its genetic alterations may render neoplastic development. MET gene amplifications in neoplasms is known to be associated with poor prognosis and resistance to anti-EGFR therapies, but is also related to a better therapeutic response to MET tyrosine kinase inhibitors, such as cabozantinib and crizotinib. Clinical trials of these drugs have currently been carried out in non-small cell lung carcinoma with MET amplification (~3% frequency) and showed promising results. To explore clinical application of MET inhibitors in other neoplasms, we screened high copy number (gene amplification or high polysomy) of MET in melanoma and variety of other carcinomas.
Design: Tissue microarrays were constructed with 19 samples of melanomas, and 116 carcinomas of various organs including lung, endometrium, bladder, pancreas and breast. MET gene copy number was evaluated by fluorescence in situ hybridization (FISH) with probes for MET gene, and CEP 7 as control (Abbott Molecular, Des Plaines, IL). MET gene was considered amplified when MET: CEP7 signal ratio was ≥2.0 or > 20 copies per nucleus of MET signals in > 10% tumor nuclei. High polysomy was defined as ≥ 4 MET gene copy number per nucleus on average.
Results: High copy number of MET were identified in 37% (7/19) of melanoma, 11% (2/19) for amplification and 26% (5/19) for high polysomy. For carcinomas, however, no MET amplification was identified, and only 8/116 (7%) of cases showed high polysomy, including 3 non-small cell lung cancers, 2 large cell neuroendocrine carcinoma of lungs, 1 small cell bladder carcinoma, 1 endometrioid carcinoma and 1 pancreatic adenocarcinoma.
Conclusions: MET high copy number (amplification/high polysomy) was identified in as high as 37% of melanoma, which is much higher than that seen in carcinomas (7%). Therefore, target therapy with MET tyrosine kinase inhibitors on melanoma apparently deserves a clinical investigation as well.

530 Can Psoriasis Have Eosinophils?

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Background: Psoriasis is a chronic skin condition, thought to be related to immune dysregulation. Numerous other conditions, including nummular dermatitis, mycosis fungoides and drug reactions (in particular to TNF alpha inhibitors) can mimic psoriasis. There is a common belief that the presence of eosinophils argues against the diagnosis of psoriasis, but actual literature is scant. We evaluated a large cohort of biopsies from psoriasis patients for the presence of eosinophils, in addition to other classic histologic features.
Design: Skin biopsies from 2013 to 2016 with a diagnosis of psoriasis, either in the final diagnosis or comment, were reviewed. To be included, the histologic features, independently reviewed by two dermatopathologists/one dermatopathology fellow, and clinical features, as reviewed by a dermatology resident blinded to biopsy results, had to both be consistent with psoriasis. Histologic parameters evaluated included dermal eosinophils (not intravascular), as well as classic histologic features of psoriasis. Any case where the diagnosis of psoriasis was in question was excluded.
Results: 85 cases met criteria: 61 psoriasis vulgaris, 14 guttate psoriasis, 8 pustular psoriasis, 1 erythrodermic psoriasis, and 1 inverse psoriasis. All cases had some degree of neutrophils in the epidermis and dilated papillary blood vessels. A diminished or complete loss of the granular cell layer was seen in 83 cases (98%) and parakeratosis was seen in 84 cases (99%). Dermal eosinophils were seen in 15 cases (18%). Of the cases with eosinophils, at most 3 eosinophils were seen upon examination of the entire dermis. Most patients had treatment for their rash prior to biopsy; in cases with dermal eosinophils 14/15 (93%) and cases without eosinophils 61/70 (87%).
Conclusions: Eosinophils are not common in psoriasis, and, when present, are a rare finding. Our findings confirm that eosinophil-rich infiltrates are not seen in psoriasis. However, the presence of rare dermal eosinophils does not exclude a diagnosis of psoriasis.

531 Diagnostic and Prognostic Value of Glucose Transporters in Melanocytic Lesions

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Background: Glucose is the primary energy source of malignant lesions. Cancer cells have increased rates of glucose uptake and metabolism, mediated by key rate-limiting factors, glucose transporters. We have previously shown that glucose transporter 1 (GLUT-1) expression is increased in melanoma compared to benign nevi, and patients whose melanoma exhibited GLUT-1 expression had a significantly lower rate of survival than patients whose melanoma was negative for GLUT-1. However, GLUT-1 expression was negative in 45% of melanomas. These tumors may express other subtypes of glucose transporters, though, little is currently known about the expression and prognostic value of other subtypes. The purpose of this study was to evaluate glucose transporter isoforms, GLUT-2 and GLUT-3, as biomarkers for the diagnosis and prognosis of melanoma.
Design: Immunohistochemical staining for GLUT-2 and GLUT-3 was used to examine a tissue microarray consisting of 110 primary melanomas, 19 melanoma metastases, and 59 nevi. A semi-quantitative scoring method was used to determine the percentage of positive tumor cells and staining intensity. A proportional odds model was used to evaluate differential GLUT-2 and GLUT-3 expression in different melanocytic lesions. Kaplan-Meier method was used to estimate disease specific survival.
Results: GLUT-2 was completely negative in all melanomas and benign nevi examined. Increased expression of GLUT-3 was more frequent in melanoma than in nevi ($p<0.0001$), and more frequent in metastatic melanoma than in primary melanomas ($p<0.001$). 36.7% of melanoma cases co-expressed GLUT-1 and GLUT-3, 19.3% of melanoma cases only expressed GLUT-1, 27.5% of melanoma cases only expressed GLUT-3, and 16.5% of melanoma cases were negative for both markers. Kaplan-Meier disease specific survival analyses indicated that patients whose melanoma exhibited high level of GLUT-3 had significantly lower survival rates than patients whose melanoma had low GLUT-3 expression ($p=0.002$), similar to the survival result based on GLUT-1 expression.
Conclusions: These findings indicate that GLUT-3 expression was up-regulated in melanomas compared to nevi, especially in those melanomas with worse prognosis. Similar to GLUT-1, GLUT-3 may serve as a useful diagnostic and prognostic marker. GLUT-2 was not expressed in melanocytes.

532 Aberrant Expression of HMB-45 in Halo Nevi

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Background: Clinically apparent development of a depigmented zone around a nevus is termed a halo nevus. Histologically, halo nevi demonstrate dense lymphocytic infiltrate associated with melanocytic nevi. The mechanism of depigmentation is thought to involve CD8+ cytotoxic T lymphocytes and the presence of IgM autoantibodies, with subsequent apoptosis of melanocytes and/or loss of melanin synthesis. Traditionally, immunostaining for human melanoma black 45 (HMB-45) has assisted in the pathological differentiation between nevus and melanoma. A gradient of HMB-45 staining with fewer positive cells or a negative reaction in the deep portion of the lesion is common in nevi, while melanomas often show homogenous expression or complete loss of expression. Occasionally we have observed halo nevi with loss of HMB-45 gradient and presence of HMB-45 positive melanocytes in the deep portion of the lesion. The purpose of this study was to elucidate the expression pattern of HMB-45 staining in halo nevi.
Design: Twenty cases of nevi with clinical halo phenomenon and histologic features of dense lymphohistiocytic infiltrate were identified. Fifteen cases of conventional nevi were used as a control group. The HMB-45 staining patterns in these cases were evaluated using light microscopy. HMB-45 gradient was considered positive when only the superficial melanocytes show positive staining. Aberrant HMB-45 expression was designated as loss of HMB-45 gradient when the superficial and deep parts both show HMB-45 positivity.
Results: Aberrant HMB-45 expression with loss of HMB-45 gradient and the presence of HMB-45 positivity in the deep portion of the lesion was observed in 10 of 20 halo nevi (50%). A gradient of HMB-45 staining was seen in most of the conventional nevi, as is consistent with published literature. Only one conventional nevus shows focal equivocally weak expression of HMB-45 in the deep dermis (6.7%).
Conclusions: Aberrant HMB-45 expression in halo nevi is not uncommon and may be a diagnostic pitfall. It is important for pathologists and dermatopathologists to be aware of this phenomenon when HMB-45 is used to help differentiate halo nevus from malignant melanoma.

533 Primary Anorectal Melanoma: A Retrospective Analysis of 30 Patients

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Background: Primary anorectal melanoma (AM) is an uncommon but aggressive melanoma subtype. Given its relative rarity, it is unknown whether conventional parameters predictive of outcome in cutaneous melanomas (CM) are also relevant in AM. Understanding the features that correlate with outcomes in AM is necessary to standardize reporting, optimize risk stratification, and enhance clinical decision making. Thus, we analyzed associations between clinical and histopathologic features and survival in a cohort of AM patients.

Design: We identified 30 patients with AM for whom we had either ≥4 years of disease free survival or disease recurrence and/or death due to disease and recorded clinical, molecular and pathologic features. Univariate Cox proportional hazards regression models assessed associations between recorded characteristics and disease specific survival (DSS).
Results: Twenty-eight patients (93%) were Caucasian and 22 (73%) were women with a median age of 64 years. Melanoma arose primarily in the rectal mucosa in 22 patients (73%), while 8 (27%) were located primarily in the anal skin. The median tumor thickness was 9.5mm (range: 1.2-40 mm). Applying tumor thickness categories for primary CM, 70% were pT4 (tumor thickness >4.0mm). Mitotic figures were identified in 97% of cases (median=17/mm^2, range: 0-152). Ulceration, lymphovascular invasion (LVI) and perineural invasion (PNI) were present in 90%, 53% and 17% of cases, respectively. *KIT* and *NRAS* mutations were identified in 30% and 10% of cases, respectively. Twenty-four patients (77%) died of disease; 6 (20%) were alive at last follow-up; and 1 (3%) died due to other causes. The median DSS from diagnosis was 19.5 months, and the 3-year survival rate was 28.2% (95%CI: 15.8-50.6%). In univariate Cox regression analysis, decreased DSS was associated with tumor thickness >4.0mm (HR=3.08, p=0.03) and LVI (HR=2.40, p=0.05).
Conclusions: In this exploratory analysis, we found that AM is a highly aggressive melanoma subtype that predominantly affects older Caucasian women. In our AM cohort, ulceration and LVI occurred more frequently than typically seen in CM. Finally, tumor thickness and LVI correlate with reduced DSS in patients with AM—reinforcing that histopathologic parameters prognostic in CM are also predictive of outcome in patients with AM.

534 Value of Phospho-S6 Immunostaining in Benign and Malignant Trichofollicular Tumorigenesis

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Background: It has been reported a unifying model for hair-follicle tumorigenesis where phosphorylation of mTOR is the common pathway. Little is known about its expression and how their biological-behavior corralates the IHC. The mTOR controls cell growth and is activated by PKB and ribosomal S6 kinase. In malignant gliomas, mTOR-inhibitors have antiproliferative/proapoptotic effects and has been suggested as a target of therapies, may be worthwhile to group hair-follicle tumors indirectly through phospho-S6 expression, since quantifying phospho-mTOR directly in fixed tissue is not feasible. We want to evaluate how phospho-S6, an activator of mTOR, is expressed in 6 different hair-follicle tumors which show different morphology.
Design: Descriptive study of 66 cases, 17 fibrofolliculomas, 20 trichoepitheliomas, 19 pilomatricomas, 1 atypical PPT, 8 trichilemmal carcinoma and 1 trichoblastic carcinoma, in a period of 16 years. The diagnosis was confirmed in H&E-stained slides. The tissues were deparaffinized and the IHC results were analyzed and scored as positive (mild +, moderate ++ and strong +++) or negative.
Results: 12/17 fibrofolliculomas were positive for phospho-S6; most cases showed a patchy distribution involving 30-50% of tumor. 9/20 trichoepitheliomas were positive with a patchy distribution involving 5-20% of tumor. 17/19 pilomatricomas were positive, staining only the basaloid cells with a multifocal distribution, involving 70-90% of tumor. Of the malignant neoplasms 8/8 cases of trichilemmal carcinoma, 1/1 of trichoblastic carcinoma and 1/1 atypical PPT were positive, diffuse and with moderate-strong intensity involving 70-90% of tumor. The staining-intensity are shown in Tables.
Conclusions: All malignant tumors showed a diffuse expression of phospho-S6 involving almost 100% of tumor, however few benign neoplasms were positive with a patchy distribution. Most pilomatricomas were positive and multifocal. These findings raise the possibility of an association of mTOR activation and tumor malignancy. Malignant hair-follicles tumors can be locally aggressive sometimes requiring adjuvant therapy. The early detection of these tumors is important and targeted therapy with mTOR inhibitors in cases demonstrating phospho-S6 expression may be feasible to avoid surgical resections with wide margins. In recurrent benign hair-follicle tumors mTOR inhibitors could be suggested as an antiproliferative drug administered after local excision, thus decreasing the recurrence of these tumors.

535 Correlation of ALK IHC with Wild-Type ALK and ALK^{ATI} mRNA Transcripts in Metastatic Melanoma

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Background: Advanced stage malignant melanoma (MM) has a poor prognosis with limited effective therapies. Recently, an ALK alternative transcription initiation (ALK^{ATI}) isoform was detected in 3-11% of MM, leading to constitutive ALK activation. MM with these alterations were shown to be sensitive to targeted therapies. Although a previous study has shown that some MM with high ALK expression by IHC typically express the ALK^{ATI} transcript, complete characterization of ALK IHC expression compared to ALK^{ATI} transcript expression has not been described. We studied a series of stage III MM patients to determine the correlation of ALK protein expression by IHC to ALK^{ATI} mRNA expression.
Design: Metastatic MM samples from 173 specimens (158 patients) with stage III disease were evaluated for ALK protein expression by IHC. In addition, mRNA was extracted from 24 tumors (13 ALK IHC positive and 11 ALK IHC negative), and 300 ng mRNA from each tumor was assessed for ALK^{ATI} and wild-type ALK (ALK^{WT}) mRNA transcript expression using NanoString technology.
Results: 13 of 173 (7.5%) MM cases were positive for ALK IHC (2 strong positive, 4 weak nuclear expression, 7 weak cytoplasmic expression, and 160 negative). The strong ALK IHC positive tumors showed nuclear and cytoplasmic staining. 62% (15/24) of cases had reliable detection of any ALK mRNA (13/13 (100%) of the ALK IHC positive cases and 2/11 (18%) of the ALK IHC negative cases). ALK^{ATI} and ALK^{WT} were the predominant transcript in 60% (9/15) and 27% (4/15) of cases, respectively. In 13% (2/15) of cases both transcripts were equally present. Strong ALK IHC positivity significantly correlated with higher ALK^{ATI} counts (p<0.0001) and increased ALK^{ATI}/ALK^{WT} ratios (p<0.002). Nuclear ALK localization by IHC significantly and directly correlated with ALK^{ATI} expression (p=0.0011), ALK^{ATI}/ALK^{WT} (p=0.0082) but not ALK^{WT} expression (p=0.6679).
Conclusions: Both ALK^{ATI} and ALK^{WT} mRNA transcripts are present in all ALK IHC-positive MM as well as in some IHC-negative MM. Higher ALK mRNA and ALK IHC expression is typically though not exclusively driven by increased ALK^{ATI} transcription. ALK positivity in MM may provide a therapeutic target for this aggressive disease, however validation through clinical studies is needed.

536 Evaluation of p16 Protein Expression and CDKN2A Deletion in Conventional and Fibrosarcomatous Dermatofibrosarcoma Protuberans

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Background: Dermatofibrosarcoma protuberans (DFSP) is a relatively common fibrohistiocytic cutaneous and superficial soft tissue tumor of intermediate malignancy. Fibrosarcomatous transformation of DFSP (FS-DFSP) is a relatively rare phenomenon and may have a far more aggressive clinical course than its conventional counterpart. Therefore, accurate histopathologic diagnosis of FS-DFSP is of crucial importance. Although limited to date, previous studies have suggested that homozygous deletion of CDKN2A gene and loss of p16 protein expression were identified in the subset of (imatinib-resistant) FS-DFSPs and these findings may be involved in the tumor progression. In this study, we analyzed a well-characterized cases of conventional DFSP and FS-DFSP for CDKN2A gene deletion and p16 protein expression.
Design: Our surgical pathology archives were searched for DFSP and FS-DFSP from 2000 to 2015. Whole tissue sections were re-reviewed. 27 cases (21 conventional and 6 FS-DFSP) were available for tissue microarray construction and examined by p16 immunohistochemistry and CDKN2A fluorescence in situ hybridization (FISH), using a commercially available antibody and probe. Appropriate controls were employed. Follow-up information was obtained from our medical records.
Results: Due to technical problems, p16 and CDKN2A FISH results were available on 17 conventional and 5 FS-DFSP. Overall, the loss of p16 protein expression was found in 7/22 (31.8%) cases; 5 conventional and 2 FS-DFSP. By FISH, one p16-negative FS-DFSP with known metastatic disease revealed a homozygous CDKN2A deletion. The remainder of conventional and FS-DFSP cases demonstrated non-specific FISH findings, including combination of monosomy 9, heterozygous CDKN2A deletion, and normal karyotype.
Conclusions: In this study, we were able to evaluate the status of CDKN2A gene and p16 protein expression in both conventional and FS-DFSP. Although homozygous CDKN2A deletion was never observed in conventional DFSP, its actual diagnostic role in the separation of conventional from FS-DFSP is unclear at this point. On-going analyses of additional cases should help to assess the exact frequencies of CDKN2A and p16 abberancy. Finally, low concordance between homozygous gene deletion and protein loss may suggest that multiple independent regulatory pathways of p16 de-activation in this tumor.

537 An Assessment of Copy Number Variations and Somatic Mutations in Advanced Melanomas by Clinical Next-Generation Sequencing

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Background: Next-generation sequencing (NGS) is routinely applied in the clinical setting to identify somatic mutations in advanced melanomas and guide patient care. Melanoma also contains frequent copy number variations (CNVs). To better characterize the mutational patterns of advanced melanomas with CNVs, we correlated the presence and frequency of CNVs (primarily copy gains / amplifications) and somatic mutations in a large cohort of advanced melanomas determined by the same NGS platform.
Design: We performed a retrospective analysis of 103 advanced (mostly metastatic) melanoma samples that underwent NGS testing at our institution between 1/2016 and 8/2016. The presence and prevalence of CNV/amplifications and somatic mutations were identified. The association between CNV and co-mutational presence was also examined.
Results: Of the 103 melanomas assessed, 19 (18%) contained CNVs detectable on our platform. Among the CNV-positive cohort, the most common CNVs identified were amplifications in *BIRC2/3* (32%), *MYC* (16%), and *MDM2* (16%). Other notable genes amplified include *NRAS* (10%), *KIT* (10%), *EGFR* (5%), *KRAS* (5%), and *TERT* (5%). Six of the 19 CNV-positive cases (32%) had 2 or more CNVs, and the majority of CNV cases (17/19; 89%) carried at least 1 somatic mutation. The most common mutations present in the CNV-positive cohort include *NRAS* (42%), *TP53* (32%), *BRAF* (26%), and *CDKN2A* (21%). Among the melanoma samples that did not have CNVs (n=84), the most common gene mutations include *BRAF* (36%), *TP53* (27%), *NRAS* (21%), *CDKN2A* (21%), and *NF1* (20%). No statistically significant associations were found between CNVs and somatic mutations in our cohort.
Conclusions: A high percentage of advanced melanoma cases with CNVs possess concurrent somatic mutations. The most common gene mutations found in CNV-positive melanomas and melanomas without identifiable CNVs are similar, and only differ slightly by mutational frequency. Due to the sometimes extreme variation in the neoplastic content of tested samples (ranging from 20 to 90%), the CNV detection frequency may be lower than expected (particularly for copy losses or deletions, but also amplifications), and thus potential associations between CNV and somatic mutations are likely not fully identified in our study. Efforts to increase the CNV identification sensitivity on our NGS platform are ongoing.

538 Expanding the Molecular Diversity of Epithelioid Fibrous Histiocytoma

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Background: Epithelioid fibrous histiocytoma (EFH) is an uncommon cutaneous neoplasm that was traditionally considered a variant of fibrous histiocytoma. This notion was challenged with the discovery these tumors harbor disease-defining rearrangements involving *ALK*. More recently, with a report of next-generation sequencing in two cases, the *ALK* fusion gene partners were reported to include *SQSTM1*- and *VCL*-. Herein, we report the results of next-generation sequencing on three additional cases of EFH.

Design: We performed a retrospective review of our archive for EFH in which cytogenetic and/or immunohistochemical testing had previously confirmed the presence of *ALK* rearrangement and/or overexpression. Using a 507 gene RNA-seq targeted fusion panel, RNA was extracted from formalin-fixed paraffin-embedded tissue. RNA-seq libraries were prepared using 20-100 ng total RNA and subsequently enriched by hybridization to the targeted fusion panel. Each sample was sequenced with 76 base-pair paired-end reads on an Illumina MiSeq at 8 samples per flow cell (~3 million reads per sample). The results were analyzed using STAR aligner and Manta fusion caller.

Results: Adequate RNA was obtained in each case. In two cases high-confidence fusion calls confirmed the presence of *SQSTM1-ALK* fusion products. In the third case a heretofore undescribed *ALK* gene fusion product was identified. Fusion products were confirmed by reverse transcription polymerase chain reaction testing.

Conclusions: Our findings, while limited, suggest *SQSTM1-ALK* may represent a common fusion gene in EFH. Interestingly, this fusion product is not unique to EFH, and has been reported in *ALK*-positive large B-cell lymphoma and non-small cell lung cancer. A pleiotropic-like relationship, whereby a fusion gene is shared amongst seemingly disparate neoplasms, is not uncommon. Indeed, it is possible other *ALK*-associated tumors may harbor this product. Finally, this study further expands the spectrum of *ALK* fusion products orising in EHF with the discovery of a previously unreported *ALK* fusion partner.

539 Tumoral and Peritumoral Immune PD-L1 Expression in Vulvar and Vaginal In Situ and Malignant Melanoma

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Background: Primary malignant melanoma (MM) of the vulva and vagina are aggressive neoplasms that present unique challenges in surgical management. Programmed death-ligand (PD-L1) expression has been associated with immune evasion and response to PD-1/PD-L1 inhibitor therapies in cutaneous melanoma but has not been investigated in vulvar/vaginal MM. We evaluated PD-L1 and CD8 in primary melanomas of the vulva and vagina.

Design: IHC for PD-L1 (SP142) and CD8 was performed on whole tissue sections of 29 cases including 13 vulvar MM, 9 vaginal MM, as well as 5 vulvar and 2 vaginal cases with melanoma in situ (MIS). In four cases we examined staining in the primary tumor and nodal metastases. Membranous staining was scored by extent in tumor cells and the peritumoral immune cells. The percent of CD8+ cells was quantified.

Results: Tumor PD-L1 staining was seen in 78% and 55% of vulvar and vaginal melanomas, respectively (Table 1). Extensive tumor staining (>25%) was rare in both vulvar (17%) and vaginal primaries (9%). Peritumoral immune staining was seen in 89% of vulvar and 72% of vaginal primaries (Table 2). Cases with larger percentages of CD8+ cells corresponded to cases with higher immune PD-L1 staining (CD8>50% of cells, p=0.0001). In cases in which nodal metastases were compared, 50% of cases showed increased expression, while the other 50% showed a relative decrease in staining. All but 1 case of MIS showed tumoral PD-L1 staining. In cases with coexisting MIS and MM an increase in PD-L1 staining in tumor and immune cells, as well as an increase in peritumoral CD8+ cells was seen in the MIS component.

Tumor Staining (PD-L1)	Vulvar MM and MIS	Vaginal MM and MIS
0	4/18 (22%)	5/11 (46%)
1: 1-5%	8/18 (44%)	3/11 (27%)
2: 6-10%	1/18 (6%)	1/11 (9%)
3: 11-25%	2/18 (11%)	1/11 (9%)
4: 26-50%	2/18 (11%)	0/11 (0%)
5: >50%	1/18 (6%)	1/11 (9%)

Immune Staining PD-L1	Vulvar MM and MIS	Vaginal MM and MIS
0: 0-5%	2/18 (11%)	3/11 (27%)
1: 5-10%	4/18 (22%)	2/11 (18%)
2: 10-50%	10/18 (56%)	6/11 (55%)
3: >50%	2/18 (11%)	0/11 (0%)

Conclusions: Vulvar and vaginal MM and MIS show frequent expression of PD-L1 in tumoral and peritumoral immune cells. These results suggest that a subset of these tumors may respond to immune checkpoint inhibitor therapy. However, diffuse positivity is rare therefore clinical trials are needed to establish staining thresholds required to predict response to PD-1/PD-L1 inhibitors.

540 Expression of PAX8 in Metastatic Melanoma

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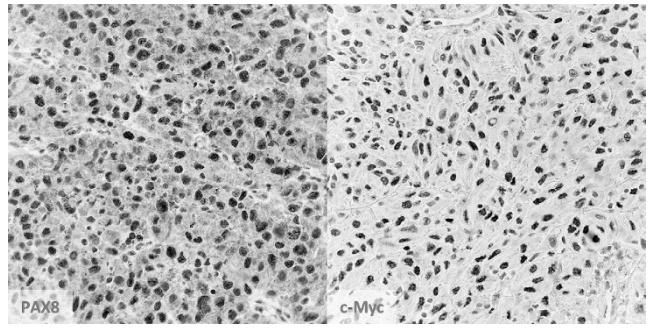
Background: Melanoma is recognized to aberrantly express immunohistochemical (IHC) markers especially in advanced or metastatic stages. PAX8 is a transcription factor marker used in metastatic tumors of unknown primary to support renal or Mullerian origin. Transcription factors like c-Myc have shown differential expression between early and advanced stage melanomas. However, PAX8 expression has not been described in melanoma. We aimed to assess PAX8 expression against c-Myc and Ki-67 in metastatic melanomas and identify potential pitfalls for using this marker in differentiating carcinomas from melanoma.

Design: Non-nodal, Stage IV metastatic melanomas from 2013-2016 were reviewed. Where available, corresponding primary tumors were also retrieved. For comparison, a nodal metastatic melanoma and atypical nevus were also stained. IHC for PAX8, c-myc, and Ki-67 was performed. PAX8 and c-Myc were scored from 0-3 as follows: 0 – negative with <1% nuclei staining, 1 – 1-25% nuclei staining, 2 – 25-50% nuclei staining, and 3 - >50% nuclei staining. The proliferation index by Ki-67 was assessed as a percentage of lesional cells.

Results: Twenty-eight cases meeting study criteria were identified with three available corresponding primary tumors. Among these, 26 (26/28, 93%) were positive for both PAX8 and c-Myc. A positive result is shown in Figure 1.



PAX8 staining was expressed to a greater extent compared to c-Myc (p=0.005). While c-Myc diminished in expression from the primary to metastatic lesion in two of the three cases (66%) with corresponding primaries, PAX8 expression increased in all of these cases. In contrast, the nodal metastatic melanoma and atypical nevus were negative for both markers. One PAX8-negative primary melanoma corresponding to a PAX8-positive metastatic tumor was identified. The Ki-67 proliferation index was not significantly associated with expression of PAX8 or c-Myc.

Conclusions: To our knowledge, we are first to report PAX8 expression in non-nodal metastatic melanomas and their corresponding primary tumors. While c-Myc expression diminishes in metastatic lesions compared to corresponding primaries, PAX8 expression increases. Our findings suggest that PAX8 is overexpressed in advanced-stage melanomas which may constitute a diagnostic pitfall.

541 Targeted NGS on Primary and Metastatic Malignant Melanoma

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Background: Metastatic malignant myeloma (MM) carries a dismay prognosis. The on-going development of targeted therapies, such as BRAF, MEK, and NRAS inhibitors, either as single or syngeneic agents, have shed light on the treatment of this old foe. Molecular diagnosis is playing more and more important roles in aiding the diagnosis, prognosis, and most importantly, personalized targeted therapy of MM.

Design: From 2014-2016, we performed next generation sequencing (MGS) molecular test on primary and metastatic MM specimens from Mount Sinai Medical System. Our targeted NGS includes 50 the most clinically significant oncogenes, tumor suppressors, and signaling molecules.

Results: Among the 67 specimens we tested, 48 patients show unique mutations that resulting in clinically known actionable alterations. We studied the frequencies of these mutations and their association with clinical features. The patients are 31 males and 17 females, the mean age is 62.2 years (range: 21-90 years). There are 28 metastatic MM and 19 primary MM. Forty-one type of mutations are detected. They belong to 17 genes. The most frequently gene involved are BRAF (20), NRAS (18), and TP53 (9). The most frequent mutations are BRAF V600E, and NRAS Q61. Only one out of two mucosal MM and none of the 5 acral MM bear cKIT mutation. Metastatic MMs are more likely to have mutations, and bear more than one mutation. Among the 10 cases of multiple mutations, 70% (7) are metastatic MM. BRAF (3) and NRAS (3) mutations are mutually exclusive in these 10 cases. About 15 cases have CAP protocol. Among them, NPAS mutation is associated with low Breslow depth (7 cases <5 mm, and 1 case > 12mm). No other mutation pattern is identified regarding staging, mitosis count, and Clark level.

Conclusions: The results demonstrate that cKIT mutation is infrequently detected in mucosal and acral MM (1/6). Metastatic MM tend to has gene mutation, and more than one mutations, therefore may render them resistance. They may benefit more from synergetic therapeutic agents. The identification of mutations in signal transduction pathways in MM provides understanding of the molecular alterations in MM. NGS or other molecular techniques should be routinely as part of the diagnosis process. The results could direct the existing targeted therapy and also identify potential new therapeutic targets for drug development.

542 The Application of *COL1A1-PDGFB* Fusion Gene Detection by Fluorescence In Situ Hybridization in Biopsy Tissue of Dermatofibrosarcoma Protuberans

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Background: Dermatofibrosarcoma protuberans (DFSP) is a dermal and subcutaneous intermediate tumour. Compared to other techniques, biopsy is not only a rapid outpatient procedure but also the optimal choice for unresectable patients with cutaneous tumor before neoadjuvant therapy. In DFSP, the rarity of tumours, atypical clinical manifestation and uncommon histological features may cause diagnosis pitfalls in the limited biopsy samples. The most remarkable cytogenetic characteristic of DFSP is the formation of the *COL1A1-PDGFB* fusion gene. The aim of this study was to analyse the clinical application value of fluorescence in situ hybridization (FISH) in biopsy of DFSP.

Design: Twenty-three consecutive biopsy specimens of DFSP, collected from 2007 to 2014, were reviewed for clinicopathological features and immunohistochemical staining results, and detected with the *COL1A1/PDGFB* Fusion Probe and the PDGFB Break Apart Probe using FISH analysis.Controls included 5 fibromatosis and dermatofibromas. The positive criteria for *COL1A1-PDGFB* gene fusion and *PDGFB* rearrangement were both 10% and more of the cells showed meaningful signals.

Results: The 23 tumour samples consisted of 10 punch biopsies and 13 shave biopsies (11 males and 12 females; mean age at diagnosis, 37 y; range, 14 to 75 y). Eighteen conventional DFSP, 1 Bednar tumour, 2 myxoid DFSP and 2 DFSP with fibrosarcomatous areas were included in the group.CD34 expression was strong and extensive in the spindle cell component in 19 cases (83%) of DFSP, including FS-DFSP, whereas one case of a conventional subtype was negative for CD34;One case of a conventional subtype and 2 myxoid subtypes had patchy CD34 expression.SMA was focal positive staining in 3 cases (13%). All cases were negative for desmin, S-100 protein, myogenin, Bcl-2, CD99, p16, p63, cytokeratin and EMA. In FISH detection, 21/23 cases (91%) were both positive for the *COL1A1-PDGFB* fusion signal and the *PDGFB* break apart signal. The percentage of *COL1A1-PDGFB* fusion gene was above 70% in 20/21 (95.2%) of DFSP. A *PDGFB* split signal pattern was observed in approximately 20%-80% of the interphase tumour nuclei.

Conclusions: This is one of the few study to demonstrate the great value of detecting *COL1A1-PDGFB* fusion translocation by both the Fusion Probe and the Break Apart Probe in biopsies of DFSP using FISH analysis. As a molecular cytogenetic technique, FISH could become a standard procedure to confirm unusual clinical presentation and validate complicated and suspected diagnosis in the routine biopsy of DFSP.

Education

543 Mentoring Residents in Academic Centers, the Cleveland Clinic Experience

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Background: Mentoring is widely recognized in academic and non-academic centers as a critical component of professional development. Even though the prevalence of mentoring in academic medical centers has been reported in the literature varying from 19 to 84%, there is limited experience in the pathology field (Zerzan J et al, Acad Med 2009). The goal of this study is to present some preliminary data on our own experience and the impact of an organized mentorship program.

Design: Our pathology department is a subspecialty based practice with more than 80 faculty members between anatomic and clinical pathology throughout the entire health system. Our ACGME accredited residency program includes a total of 29 PGY1-PGY4 residents. A pilot mentorship program was introduced to residents and staff volunteers, which included a training session that provided educational material, as well as basic guidelines and exercises examples on how to make the most out of the mentorship experience. Baseline and follow up surveys were obtained.

Results: Our pilot program included 19 staff (2 to >25 years in practice) and 20 residents (12 female, 8 male, PGY1 to PGY4). The baseline survey revealed that >85% of residents were interested in having a mentor. Even though 37.5% reported having at least 1 mentor before starting the program, 4 out of 5 residents struggled to find a mentor during the first year of training. Out of the entire cohort, the mentor-mentees match was successful in 94% of cases. Anecdotal feedback as well as follow up surveys from residents reported that mentors could help them achieve their goals, including development of career objectives (86%), fellowship/job search (67%) and overcoming daily obstacles (40%).

Conclusions: Our experience supports that mentor-mentee relations are greatly valued and key in professional development. Besides spontaneous mentoring, an organized mentorship program may be helpful as an additional tool to facilitate interactions early during residency and to support staff and residents through educational resources.

544 A Survey of Pathologists and Learners of Pathology on Pathology Information Consumption

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Background: Observational data suggests seekers of pathology information (PI) have focused questions and value the ease of finding info, based on web statistics; however, PI consumption is still incompletely characterized and infrequently stratified by practicing pathologist and learner. Where information is sought and what is important may give insight into how PI may be delivered in the future.

Design: An online survey was done using limesurvey (limesurvey.org). Participants were recruited from an open access pathology web site, via Twitter, email and word of mouth.

Results: A total of 59 participants completed the survey (25 pathologists, 33 learners (3 fellows, 30 residents), 1 other health professional) and were from various regions (North America 39, Asia 9, Europe 5, Africa 5, Other 1). Among learners (L) and pathologists (P) elements rated very important (VI) were images (70% L/60% P), followed by microscopic criteria (58% L/56% P) and IHC info (52% L/56% P). Learners and pathologists differed on the VI ratings for spot diagnosis quizzes (39% L/28% P), sign out examples (45% L/28% P), image annotations (27% L/48% P) and references (12% L/28% P). Both groups very frequently (VF) sough info via search engines (30% L, 32% P) and open access web sites not involved in the survey (42% L/36% P). Learners preferred login web sites (24% vs 12% VF) and colleagues (30% vs 16%), while pathologists preferred the primary literature (36% vs 6%) and review articles (24% vs 6%). Offline textbooks (28% vs 21%) and Wikipedia (16% vs 9%) were more VF used by pathologists; however, learners less frequently 'never' used Wikipedia (16% vs 6%).

Conclusions: The interest in images suggests that picture-matching is important and images showing variation likely desired. The interest in sign out examples among learners may indicate an increased desire for standardization. The differing importance placed on references and the medical literature may reflect a change in where individuals get information (media vs social media) and the ease of finding information/verification with other sources. PI seekers use a variety of sources. Offline resources remain important; however, learner-pathologist differences suggest that PI is increasingly being sough online and open access resources may be preferred.

545 Improving Patient-Pathologist Interactions Using Structured Communication Training for Pathologists- A Pilot Experience

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Background: Patients occasionally request communications with pathologists. Pathologists express discomfort or avoid direct patient interactions, as these are often triggered by negative events. If not handled with sensitivity, transparency and concern for the patient, these may cause greater distress for the patient, and negative outcomes.

Design: As part of the institutional communication and resolution program, several pathologists underwent structured communication training. This pilot evaluated comfort and quality of patient-pathologist conversations before and after training. Reports of patient experiences were collected from clinicians who interacted with the patients after the disclosure conversation.

Results: There were 5 communication events. 4 generated from erroneous reports, 1 of which led to a delay in diagnosis. 1 was a complex clinico-pathologic event. 2 involved untrained pathologists. 2 (1 before and after) involved the same pathologist. The quality of the conversations, as reported by pathologists and the patients, was better and more comfortable with a pathologist with training/coaching. Both patients who communicated with untrained pathologists expressed dissatisfaction with the communication. One reported the pathologist as insensitive and unsympathetic. Untrained pathologists were apprehensive, felt ill-equipped for the meeting and reported that patients did not appear to understand the complexity of pathologic diagnoses. They did not offer patients the opportunity for follow-up conversations. They were disinclined to participate in additional patient interactions. Patients who interacted with trained/coached pathologists expressed acceptance (1) and satisfaction for the transparency and quality for communication (1). Trained pathologists expressed greater comfort going into the meeting and navigating the discussion, and offered follow-up opportunities to the patients. They independently followed up with the patient (1) and the clinician (1).

Conclusions: Difficult conversations are a necessary part of a physician's life. They are likely to increase in frequency for pathologists, because of greater access to the pathology report for patients, and increasing involvement of the pathologist by the clinician in disclosure conversations. Communication training for pathologists may aid the pathologist in more comfortably and empathetically navigating conversations with patients and result in a more positive experience for the patient and the pathologist.

546 Cytology Cases of the Week: Five Years of Data on an Educational Tool That Improves Trainee Exposure to Cytology

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Background: Cytology is a major subspecialty within anatomic pathology, accounting for ~30% of the "practical with images" section of the anatomic pathology board exam. Further, the ACGME requires residents to examine 1,500 cytology specimens by the end of residency. Cytology cases of the week (COWs) were instituted in 2011 in an effort to increase trainee exposure to cytology. Here, we assess the effectiveness of COWs as a teaching tool.

Design: Images of 2-5 cases/week with basic clinical information are sent to residents on a weekly basis. Residents have one week to respond by email with diagnoses; after which, correct answers are emailed. Cytology resident in-service examination (RISE) scores were compared between participants in COWs and non-participants from 2011-2015. In addition, an anonymous survey was distributed to trainees using Qualtrics Survey Software to determine how often residents participated in COWs, reasons hindering or promoting participation, and perception of the effectiveness of COWs as a teaching tool.

Results: An unpaired two-sided t-test showed residents who participated in COWs scored 15.4% higher on the RISE than residents who participated minimally or not at all ($p<0.05$) over the 5-year period. Analysis of the years separately showed residents who participated in COWs on average had higher RISE scores than non-participants (6.7-25.2%), and this difference was statistically significant in 3 of 5 years ($p<0.05$). In 2016, COWs were not sent. An unpaired t-test comparing RISE scores for residents