Design: 421 archived cases of EC(1995-2007) were reviewed and TMAs prepared as per established procedures. ERCC1 and RRM1 Immunofluorescence stains were combined with Automated Quantitative Analysis to assess their expression. The average of triplicate core expression was used to determine high and low score cutoff points using log-rank test on overall survival(OS). Association between expression profiles and clinicopathological parameters was tested using Fisher's exact test. The independent prognostic value of ERCC1 and RRM1 was tested using Cox model adjusted for traditional prognostic factors.
Results: 304(72%) type-I EC cases and 117(38%) type-II EC cases were identified. Caucasian women had higher proportion of type-I tumors($p<0.001$) while elderly women were more likely to have type-II tumors ($p<0.001$). ERCC1 and RRM1 expression was observed in 80% of tumors (336 cases & 335 cases,respectively). Kaplan Meier curves showed statistically significant difference in OS between low and high expression of ERCC1 and RRM1. OS remains significantly different using Cox model adjusted for other covariates (age, race, histologic subtypes, lymph vascular invasion and stage) for the two markers. High ERCC1 scores were associated with increased OS when compared to low ERCC1 scores($p=0.007$). In contrast, low RRM1 scores were associated with better OS compared to higher RRM1 scores ($p=0.007$). Log-rank test demonstrated that type-I tumors and advanced stage (FIGO III and IV) tumors could each be subdivided further into better and worse survival groups based on low and high RRM1 expression respectively($p<0.006$). Similarly, lower stage (FIGO I and II) tumors with higher ERCC1expression are associated with better overall survival that those with lower ERCC1 expression($p=0.026$).
Conclusions: We found ERCC1 and RRM1 to be independent prognostic factors for overall survival of EC. They could be utilized for possible future molecular classification and help to tailor optimal individual therapy.

1295 Single PIK3CA Hotspot Mutation Detected in Cervical Squamous Cell Carcinoma: Implications for Targeted Therapy

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Background: The treatment of uterine cervical squamous cell carcinoma (SCC) after surgery is limited to radiation therapy with or without cisplatin-based chemotherapy. Although targeted therapy has been widely explored in many other tumors, it has not been applied to cervical SCC due to lack of data on cancer-specific mutations.
Design: Cancer Gene Mutation Panel with targeted next generation sequencing was performed on 33 cases of cervical SCC to target 2,855 clinically actionable mutations in 50 key cancer genes. The patients ranged from 30 to 80 years of age (mean = 52 years). They all had invasive cervical SCC greater than 0.5cm to ensure adequate DNA extraction. Sequencing libraries were obtained in 28 of the 33 cases with 5 cases failed library preparation due to poor DNA quality.
Results: Seven of the 28 cases (25%) harbored a single point mutation in phosphatidylinositol 3-kinase, catalytic subunit (*PIK3CA*). Six of the seven cases had a single *PIK3CA* E545K mutation and one had a single *PIK3CA* Q546R mutation. Of note, none of the 28 cases had any other hotspot mutation among the remaining 49 genes in the panel, including HPV infection-associated genes such as *TP53, RB1* and *NOTCH1*.
Conclusions: Cervical SCCs harbor a high rate of oncogenic *PIK3CA* mutations. The data strongly suggest that the single point mutations of PI3KCA may drive the carcinogenesis of cervical SCC. More importantly in an era of increasing precision medicine, PI3KCA is potentially targetable for personalized cancer therapy with PI3K inhibitors.

1296 GATA3 Is Expressed in Vulvar Paget's Disease

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Background: GATA-binding protein 3 (GATA3) is a zinc-finger transcription factor involved in cell development and differentiation. Recent studies have shown that GATA3 is a useful marker for breast and urothelial carcinomas. Vulvar Paget's disease (PD) is uncommon but it may show invasion and metastasize. The invasive adenocarcinomatous component associated with vulvar PD shows similar morphology to breast carcinoma and poses diagnostic confusion with the latter in metastatic sites. Here we investigated the status of GATA3 in vulvar PDs with immunohistochemical staining (IHC).
Design: Twenty-four vulvar PDs were included: 6 with an invasive adenocarcinoma component and 18 without. One representative tissue block from each case was used to generate 4um unstained slides for IHC with GATA3. We also stained estrogen receptor (ER), progesterone receptor (PR), and gross cystic disease fluid protein 15 (GCDFP 15) in these tumors for comparison. The staining was semi-quantitatively scored as negative (no tumor cells stained), 1+ (1-25%), 2+ (26-50%), 3+ (51-75%), and 4+ (76-100%).
Results: All 18 PDs without invasivion showed positive GATA3 staining including 1+ in 1, 2+ in 1, 3+ in 1 and 4+ in 15. Positive GCFDP15 staining was seen in 15/18 (83%) PDs including 1+ in 8, 2+ in 3, 3+ in 1, and 4+ in 3. GATA3 stained more tumor cells than GCDFP15 in 11, similarly in 3 and less than GCDFP15 in 1 case, respectively. Only 1/18 PDs was focally positive for ER (1+) and all PDs were negative for PR. All 6 PDs with invasion showed positive GATA3 staining, including 5 positive in both in situ (all 4+) and invasive components (2+ in 1, 4+ in 4) and 1 positive only in the in situ component (1+). GCDFP15 staining was seen in 5/6 cases, including 4 in both components (in situ: 1+ in 1, 3+ in 2, 4+ in 1; invasive: 1+ in 1, 4+ in 3) and 1 only in the in situ component (1+). In the 6 invasive components, 3 showed GATA3+/GCDFP15+ (GATA3 stained more cells than GCDFP15 in 1, similar to GCDFP15 in 1, less than GCDFP15 in 1), 2 showed GATA3+/GCDFP15-, and 1 showed GATA3-/GCDFP15+. One case was positive for both ER and PR in both in situ and invasive components (in situ: 1+ for ER and PR; invasive: ER 4+ and PR 2+) and the remaining 5 cases were negative for both ER and PR.
Conclusions: Positive GATA3 staining is seen in all vulvar PDs. GATA3 staining is generally retained in the invasive component associated with vulvar PDs. GATA3 is more sensitive than GCDFP15 for vulvar PDs. Vulvar PDs only rarely express ER and PR. Vulvar PD should be added to the GATA3+/GCDFP15+ tumor list.

Head and Neck Pathology

1297 Subclassification of Perineural Invasion in Oral Squamous Cell Carcinoma: Prognostic Implications

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Background: Perineural invasion (PNI) is an established independent predictor of adverse outcome in many malignancies including oral squamous cell carcinoma (OSCC) and often results in escalation of treatment. However, detailed histologic analysis and subcategorisation of PNI to select the cohort most at risk has not been attempted.
Design: Clinicopathologic data of OSCC patients were extracted from a prospectively collected database (1995-2012) at a single institution. The Pathology was reviewed for tumor differentiation, tumor depth, patterns of invasion (POI), PNI, lymphovascular invasion (LVI), bone invasion and margin status. The parameters of PNI assessed included: a)uni or multifocal, b)measurement of the size of the involved nerve, c)the location of the involved nerve as intratumoral, at the advancing tumor front, or beyond with measurement of the distance from the tumour for those beyond. Statistical analyses included Chi square test, Kaplan-Meier method, Cox regression analyses.
Results: The study includes 363 patients with OSCC (M:F 223:140, median age 64y, median follow up 6y). PNI was seen in 99 (27%) patients. Presence of PNI correlated significantly with local failure ($p=0.046$) but not with disease specific survival (DSS), regional or distant metastases. On multivariate analysis multifocal PNI (HR:8.7, 95%CI:1.1-70, $p=0.042$) and size of involved nerve (>1mm) (HR:4.9, 95%CI:1.3-18, $p=0.016$) were significantly associated with local failure. Of the 99 patients with PNI, 49 (49%) also showed LVI, bone invasion, or involved margins. 64 (64%) patients received radiotherapy (RT). The use of adjuvant RT amongst patient with PNI did not result in significant differences in the rate of local control, DSS, regional or distant metastases.
Conclusions: The data from this well characterised cohort with a long follow up indicate that presence of multifocal PNI or involvement of nerves >1mm is a significant predictor of local failure. While pathology reports may comment on multifocality of PNI, the size of the nerve is rarely measured. Objective inclusion of these parameters in reports would facilitate larger studies and correlation with clinical outcome.

1298 Significantly Increased Pepsin in Vocal Fold Squamous Cell Carcinoma: An Implication of Carcinogenic Effects of Chronic Laryngopharyngeal Reflux

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Background: Pepsin is primarily synthesized by the chief cells in the stomach as a pro-form zymogen, pepsinogen. Upon being released into the acidic environment of the stomach, pepsinogen is converted into the active form, pepsin, a digestive protease. The larynx is exposed to pepsin, present in the gastric content, following episodes of laryngopharyngeal reflux (LPR). The pepsin will then be internalized by laryngeal epithelial cells by the process of receptor-mediated endocytosis. It has been well established that the presence of pepsin in the upper aerodigestive tract is a sensitive and specific marker for laryngopharyngeal reflux. It has been hypothesized that the presence of pepsin in the larynx could lead to mucosal damages, inflammation and promotion and head and neck carcinogenesis.
Design: A total of 20 cases of vocal fold SCC and 8 cases of benign vocal fold lesions (polyp and keratosis) were retrieved from the Department of Pathology, Central Arkansas Veterans Healthcare System, Little Rock, Arkansas and analyzed by immunohistochemical (IHC) staining using a mouse monoclonal antibody against pepsin (Acris Antibodies, San Diego, CA). The intensity of the cytoplasmic pepsin immunostain was semiquantitatively scored as follow: negative to week (0 to 1); week to moderate (1 to 2); moderate to strong (2 to 3) and strong (3). The individual scores will then be averaged and comparison between vocal fold SCC and benign lesions is made with student's T Test.
Results: Significantly increased pepsin was detected in vocal fold SCC as compared to benign vocal fold lesions. The amount of pepsin as reflected by the IHC staining intensity for vocal fold SCC ranged from 1.5 to 3 with an average score of 2.4 while that for benign vocal fold lesions ranged from 1 to 2 with an average score of 1.25. The difference in the pepsin IHC staining intensity between vocal fold SCC and benign lesions is statistically very significant ($p < 0.001$).
Conclusions: the detection of increased pepsin in most cases of vocal fold SCC supports the co-existence of LPR in these patients and strongly implies that pepsin or chronic LPR may play important role in the carcinogenesis of vocal fold SCC, either alone or more commonly in collaboration with drinking and cigarette smoking.

1299 Promoter Hypermethylation of SOCS1 Gene, a Negative Regulator of EGFR-Signaling Pathway, Significantly Reduces the Survival in Patients with HNSCC

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Background: Epidermal Growth Factor Receptor (EGFR) signaling pathway appears critically important in head and neck squamous cell carcinoma (HNSCC) progression.

For this reason, there has been immense interest in exploring targeted therapies aiming at EGFR, using either EGFR tyrosine kinase inhibitors (EGFR-TKI) or anti-EGFR monoclonal antibodies (EGFR-mab) for HNSCC. Suppressor of cytokine signaling 1 (SOCS1) is an effective, negative regulator of the EGFR signaling pathway. Characterization of epigenetic regulation of SOCS1 may lead to more effective treatment of HNSCC using EGFR-targeted therapies.
Design: A total of 51 cases of primary HNSCC were retrieved from the Department of Pathology with comprehensive clinical follow-up. Genomic DNA samples from these 51 cases were extracted, modified with sodium bisophite, followed by PCR amplification using methylation-specific primer set and agarose gel electrophoresis. Overall patient survival was calculated using the Kaplan-Meier method. Multivariate analysis was performed using a Cox regression model.
Results: Among 51 cases of primary HNSCC, 10 (19.6%) displayed promoter hypermethylation of the SOCS1 gene. By Kaplan-Meier method, SOCS1 promoter hypermethylation in tumor showed statistically significant association with decreased overall 5-year survival ($p = 0.007$), cause-specific survival ($p = 0.024$) and 2-year disease-free survival ($p = 0.046$). Five-year overall, cause-specific and 2-year disease-free survival were 0%, 0% and 12.3% for HNSCC with SOCS1 promoter hypermethylation and were 17.5%, 16.6% and 47.5% for those without SOCS1 promoter hypermethylation. Multivariate analysis using a Cox regression model indicated that SOCS1 promoter hypermethylation remained to be significantly associated with patient survival in HNSCC, independent of other potential prognostic factors, such as tumor size, nodal status and clinical stage.
Conclusions: SOCS1 promoter hypermethylation is a strong and independent predictor for decreased HNSCC patient survival. Since SOCS1 is a negative regulator of EGFR signaling pathway, combined treatment modalities using DNA Demethylating agent (5-azacytidine), and EGFR-targeted agents may hold great promise to be more effective in killing HNSCC with much improved patient survival.

1300 Tubular Variant of Basal Cell Adenoma Shares Immunophenotypic Features with Normal Intercalated Ducts and Is Closely Related to Intercalated Duct Lesions of Salivary Gland

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Background: Intercalated duct lesions (IDLs) of salivary glands are characterized by proliferation of closely apposed intercalated ducts and show a variety of patterns ranging from hyperplasia to adenoma. Among the salivary tumors, basal cell adenoma (BCA) seems to be the most frequently associated with IDL, leading to the hypothesis that IDL could be a precursor of BCA. BCAs show a variety of histological patterns and the tubular variant is the one that presents stronger resemblance with IDLs. The aim of this study was to analyze the morphologic and immunohistochemical profiles of IDLs and BCAs classified into tubular and non-tubular subtypes (T-BCA and NT-BCA) to verify whether IDL and T-BCA would represent distinct entities. Although both are benign lesions with probably similar behavior, the study of their possible relationship can contribute to understanding of their pathogenesis and improve diagnostic accuracy.
Design: Eight cases of IDLs, 9 T-BCA (tumors with 80% or more of tubular pattern) and 19 NT-BCA were studied. NT-BCA subgroup was composed of the variants trabecular-tubular, trabecular, and solid. All cases were stained with CK7, lysozyme, DOG1,CK14, α-SMA, calponin, p63 and S-100. Immunoreactivity for each antibody was classified as absent (0% to 5%), focal (>5% to 50% of cells) and diffuse (>50%).
Results: All T-BCAs contained IDL-like areas, which represented around 20% to 70% of the tumor and were formed of ductal structures separated by minimal intervening stroma that blended imperceptibly with those surrounded by more than one layer of myoepithelial cells. In NT-BCA, IDL-like areas were occasional and small (< 5%). One patient presented IDLs, T-BCA and IDL/T-BCA combined lesions. Luminal ductal cells of IDLs and T-BCA exhibited positivity for CK7, lysozyme, S100 and DOG1. In NT-BCA group, few luminal cells exhibited such immunoprofile; they were mainly CK14 positive. Basal/myoepithelial cells of IDLs, T-BCA and NT-BCA were positive for CK14, calponin,α-SMA and p63; they were more numerous in BCA lesions.
Conclusions: IDL, T-BCA and NT-BCA form a continuum of lesions where IDLs are closely related to T-BCA. In both, the immunoprofile of luminal and myoepithelial cells recapitulates normal intercalated duct. The difference between adenoma-like subset of IDLs and T-BCA rests mainly on the larger numbers of myoepithelial cells in the latter. Our findings indicate that at least some BCA can arise via IDL.

1301 Clincopathologic Correlation of Partial P16 Staining in Oropharyngeal Squamous Cell Carcinoma

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Background: Human papilloma virus (HPV) related oropharyngeal squamous cell carcinoma (OPSCC) has highly favorable relative prognosis. p16 staining of tumor tissue by immunohistochemistry (IHC) is a surrogate marker for HPV infection and >75% p16+ cells or presence of >50% p16+ cells with >25% confluence (groups of 10 cells) is suggested as a criteria for defining HPV+ status. However, clinical validation of these criteria or significance of partial p16 staining has not been reported.
Design: Archived tumor samples from 174 patients with OPSCC treated with curative intent from 1990-2010 were retrospectively stained for HPV-status either by IHC or in-situ hybridization. Of 81 samples stained with IHC, percentage of p16+ cells were categorized as ≤25% (36%), 26-75% (19%) or >75% (43%) patients. Percent confluent cells were categorized as ≤25% (30%), 26-75% (7%) or >75% (63%) patients. Estimates of recurrence-free survival (RFS) and overall survival (OS) were calculated. Log-rank test was used for univariate analysis.
Results: The median patient age was 58 years. Primary tumor site was base of tongue in 30 (37%) patients, tonsil in 42 (52%) and soft palate/pharyngeal wall in 9 (11%). Using reported criteria, 48 (59%) patients were labeled p16+. With a median follow up of 3.4 years, 5-year OS and RFS for p16+ patients (80% and 76% respectively) were significantly better than for p16- patients (39% each), $p < 0.001$. Based on percent p16+, 5-year RFS for ≤ 25%, 26-75% and >75% patient cohorts were 41%, 58% and 81% respectively, $p < 0.001$ [figure 1]. Based on percent confluence, 5-year RFS for ≤ 25%, 26-75% or >75% patient cohorts were 37%, 26% and 77% respectively, $p < 0.001$ [figure 2]. Using a criteria of either >75% p16+ cells or >75% confluence, 51 (63%) patients would be labeled as p16+ with 5-year RFS for the positive and negative cohorts of 77% and 34% respectively, $p < 0.001$.

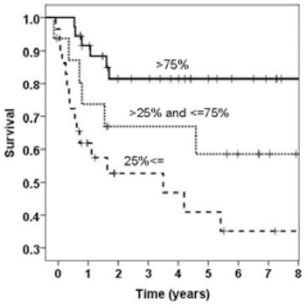

Figure 1: RFS by percentage of cellular staining for p16

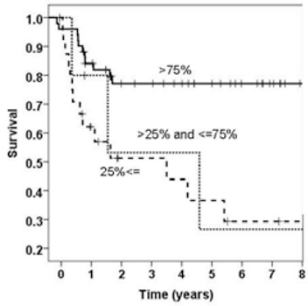

Figure 2: RFS by percentage confluence of p16 stain

Conclusions: This report clinically validates the prognostic significance of graded p16 staining and percent confluence as surrogate markers of HPV status. In cases where percentage of cells stained is less than 75%, increasing the confluence criteria for HPV positivity from >25% to >75% may improve patient dichotomization.

1302 Chondrosarcomas of the Head and Neck: A Clinicopathologic Study of 30 Cases

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Background: Chondrosarcomas (CS) arising in the head and neck are rare, comprising ~15% of all CS. Definitive management guidelines for this anatomic subset do not exist. This study was undertaken to examine the histopathologic features, prognosis and current treatment of this rare entity.
Design: A 25-year retrospective review at two separate institutions yielded 30 cases with slides available for review. Clinical and radiologic characteristics were retrieved via the electronic medical record. H&E stained slides for each case were reviewed by a specialist in bone and soft tissue pathology for histologic features including grade, myxoid change, mitotic count, bone invasion, and margin status. Clinical characteristics for each patient included demographics, radiologic features, presenting symptoms, smoking or radiation history, treatment and outcome.
Results: Demographics were as follows: M:F ratio of 1:1 with a mean age of 47 years (range 10-85). Eight patients were smokers, and 2 had a history of radiation therapy to the affected site. The primary tumor sites were: 11 skull base, 6 clival, 5 larynx, 6 nasal, and 2 maxilla. Tumor size averaged 4.5 cm (range 1-14). 14 (47%) cases were grade 1, 12 (40%) grade 2, and 4 (13%) grade 3. 15 CS contained myxoid stroma. Mitotic counts≥1/10 HPF were found only in grade 3 CS (3 cases). 20 tumors infiltrated bone. Seven patients underwent biopsy only (including one FNA), 10 marginal excision, 3 wide excision and 10 composite resections. Follow-up was available for 23 patients (mean 35 months, range 10-176). 12 (40%) patients were alive with no evidence of disease, of whom 7 had surgery alone and 5 had surgery and radiation (6 grade 1, 5 grade 2, 1 grade 3). Nine (30%) patients were alive with either stable residual disease or indolent local recurrence, all of whom underwent surgery and radiation (6 grade 1, 3 grade 2). One patient died of other causes with no evidence of disease. No patients developed metastases. Only one patient (with a grade 3 nasal CS) died of disease.
Conclusions: CS of the head and neck have a favorable prognosis following either surgery alone or in combination with radiation. Of those patients treated only with surgery, none developed recurrence. One patient with grade 3 CS died of locally aggressive disease. These findings support the alternate designation "atypical cartilaginous tumor" (for grade 1 CS), as recently suggested by the WHO.

1303 Glandular Odontogenic Cysts Consistently Lack the MAML2 Rearrangements That Are Frequently Found in Central Mucoepidermoid Carcinomas

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Background: Glandular odontogenic cyst (GOC) is a rare cyst of the gnathic bones characterized by squamous and glandular differentiation. The histopathologic features of GOC overlap with central mucoepidermoid carcinoma (MEC), suggesting that GOC could be a precursor to or low-grade form of central MEC. Differentiating the two tumors may be difficult or impossible, particularly on a limited biopsy. *MAML2* rearrangements have been recently found to be specific for MEC, even those arising in the jaws. An analysis of *MAML2* in GOCs could help clarify its relationship with central MEC.
Design: Tissue blocks from 21 GOCs and 5 central MECs were retrieved from the surgical pathology archives of The Johns Hopkins Hospital. The slides were re-reviewed to confirm the diagnoses. Each MEC exhibited solid areas and clear-cut stromal invasion. In addition, 4 of the MECs demonstrated cystic areas that were

histologically similar to GOC. Break-apart fluorescence in situ hybridization (FISH) for *MAML2* was performed. For the MECs, analysis was performed on both the solid and GOC-like cystic components.
Results: *MAML2* rearrangements were identified in all 5 of the MECs, but in none of the 21 GOCs (100% vs 0%; p <.0001, Fisher's Exact). In the MECs, the rearrangement was present in both the solid and GOC-like cystic areas.
Conclusions: While central MECs consistently harbor the *MAML2* rearrangement, even in those low grade cystic areas that resemble a pre-existing GOC, true GOCs do not. Accordingly, GOC does not appear to represent an early or low grade form of central MEC, but rather an unrelated lesion. For difficult cystic lesions of the gnathic bones where the disctinction of MEC and GOC is critical, *MAML2* FISH may be useful in aiding this distinction.

1304 Mucoepidermoid Carcinoma Does Not Harbor Transcriptionally Active High Risk Human Papillomavirus Even in the Absence of the MAML2 Translocation

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Background: High risk human papillomavirus (HPV) is now firmly established as an important cause of a subset of head and neck squamous cell carcinomas. Recent studies using polymerase chain reaction (PCR) methods have also implicated HPV as a cause of mucoepidermoid carcinoma (MEC) – a tumor of salivary gland origin that frequently harbors the MAML2 translocation. We performed RNA in situ hybridization for E6/E7 mRNA transcripts on a large group of genetically characterized MECs to establish the prevalence of transcriptionally active HPV, and to determine whether transcriptionally active HPV obviates the need for the MAML2 translocation.
Design: A tissue microarray (TMA) containing 92 mucoepidermoid carcinomas was constructed. Break-apart fluorescence in situ hybridization for MAML2 was performed on the TMA to determine the translocation status of each individual tumor. HPV testing was also performed using RNA in situ hybridization targeting HPV mRNA E6/E7 transcripts (a cocktail of 18 high risk HPV genotypes including 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, and 82).
Results: MAML2 FISH was successfully performed on 75 MECs, and 56 (75%) were positive for the rearrangement. In the presence of readily identifiable signals in HPV-positive controls run in parallel, none of the 92 (0%) MECs were positive for high risk HPV by RNA in situ hybridization.
Conclusions: The complete absence of transcriptionally active HPV suggests that HPV does not play any substitutional role for the MAML2 translocation or any role whatsoever in the development of MECs.

1305 Evaluation of PAX2 and PAX8 Expression in Salivary Gland Neoplasms

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Background: PAX2 and PAX8 are transcription factors involved in embryogenesis that have been utilized as immunohistochemical indicators of tumor origin. Specifically, PAX2 is a marker of neoplasms of renal and Müllerian origin, while PAX8 is expressed by renal, Müllerian, and thyroid tumors. While studies examining these transcription factors in a variety of tumors have been published, data regarding their expression in salivary gland neoplasms are limited. The goal of this study was to assess expression of PAX2 and PAX8 in a large cohort of salivary gland tumors.
Design: Tissue microarrays (TMAs) containing samples of 442 benign and malignant salivary neoplasms and 68 normal salivary glands were stained using antibodies against PAX2 and PAX8. Staining was scored on the basis of nuclear positivity as follows: 1+ for 1-10% positive nuclei, 2+ for 10-50% positive nuclei, and 3+ for≥51% positive nuclei.
Results: Positive staining with PAX2 among evaluable samples was as follows: 0/131 mucoepidermoid carcinomas (MECs), 1/32 acinic cell carcinomas (ACCs), 0/37 adenoid cystic carcinomas (AdCCs), 0/47 salivary duct carcinomas (SDCs), 0/18 polymorphous low-grade adenocarcinomas (PLGAs), 0/6 myoepithelial carcinomas, 0/6 epithelial-myoepithelial carcinomas (EMCs), 0/4 basal cell adenocarcinomas, 0/16 Warthin tumors, 0/19 pleomorphic adenomas (PAs), 0/7 myoepitheliomas, 0/18 basal cell adenomas, 1/53 oncocytomas, and 0/19 canalicular adenomas (CAs). Both the ACC and oncocytoma with positive staining had focal, weak reactivity (1+). Results with PAX8 were: 0/131 MECs, 0/33 ACCs, 0/39 AdCCs, 0/46 SDCs, 0/18 PLGAs, 0/6 myoepithelial carcinomas, 0/6 EMCs, 0/4 basal cell adenocarcinomas, 0/16 Warthin tumors, 0/19 PAs, 0/7 myoepitheliomas, 0/18 basal cell adenomas, 0/53 oncocytomas, and 0/19 CAs. Among normal salivary gland samples, there was evaluable tissue from 49 parotid glands, 15 minor salivary glands, three submandibular glands, and one sublingual gland, all of which were negative with both PAX2 and PAX8.
Conclusions: PAX2 expression is not seen in normal salivary glands and is very rare in salivary gland neoplasms, in which positivity appears to be focal and weak. No normal salivary gland or salivary gland tumor demonstrated expression of PAX8 in our cohort. In conjunction with previously published data, these results suggest PAX2 and PAX8 immunohistochemical stains would be useful in distinguishing salivary gland neoplasms from metastasis of morphologically similar tumors such as renal or thyroid carcinomas.

1306 Sinonasal Carcinomas: A 10-Year Review in a Tertiary Care Center

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Background: Sinonasal carcinomas include squamous cell carcinoma (SCC), adenocarcinoma, sinonasal undifferentiated carcinoma (SNUC), and salivary gland type tumors which include adenoid cystic carcinoma (ACC), mucoepidermoid carcinoma (MEC), and, very rarely, acinic cell carcinoma. They are all uncommon neoplasms, accounting for 3-5% of upper respiratory tract neoplasms. There are only a few studies comparing stage, age and localization among different histologic subsets of sinonasal carcinomas.
Design: We retrospectively studied 10 years of resections of sinonasal carcinomas and reviewed age, gender, histologic type, and stage. Tumors were classified by histologic type. For non-keratinizing carcinomas that were difficult to categorize, immunohistochemical stains were used with SCC as CK5/6 or p63 positive and SNUC as CK5/6 and p63 negative, pan-cytokeratin positive, and negative for endocrine markers.
Results: 161 resections of carcinomas involving the sinonasal tract were performed, with a slight male predominance (54%). There were 58 primary sinonasal carcinomas: 23 (14%) originated in the maxillary sinus cavity, 25 (15%) in the nasal cavity, and 10 (6%) in the ethmoid or frontal sinuses. The remaining 103 cases were primary carcinomas from the oral cavity (91), nasopharynx (6), and skin (6) with direct extension into the sinonasal tract. Patients with primary carcinomas arising in the nasal cavity or paranasal sinuses were significantly younger than those extending from the outside the sinonasal tract (p=.001). Tumors arising in the nasal cavity were less likely to present at an advanced stage than those arising in the paranasal sinuses (p=.001). 75% of tumors extending from outside the sinonasal tract were keratinizing squamous cell carcinomas, and the majority of the remainder were non-keratinizing squamous cell carcinomas or salivary gland type tumors. Primary carcinomas of the paranasal sinuses were more diverse: 31% non-keratinizing SCC, 29% keratinizing SCC, 15% SNUC, 11% salivary gland type tumors, 7% non-keratinizing SCC arising from Schneiderian papillomas, and 7% adenocarcinomas NOS. There were no cases of small cell/neuroendocrine carcinomas.
Conclusions: Sinonasal carcinomas are uncommon neoplasms of the nasal cavity and paranasal sinuses. Sinonasal carcinomas can be asymptomatic and often present at late stages. We found that primary carcinomas of the paranasal sinuses present at a later stage than primary carcinomas of the nasal cavity. These patients also tend to be younger than those with primary carcinomas with direct extension into the sinonasal tract.

1307 Keratocystoma: Three Cases of a Rare Parotid Gland Tumor

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Background: Keratocystoma is a rare tumor of the parotid gland consisting of multiple cystic spaces lined by keratinizing squamous epithelium lacking atypia. Since the original description of the lesion as a choristoma of the parotid gland by Seibert et al. in 1999, few additional cases of keratocystoma have been identified within the literature. We describe a series of three cases of this unusual tumor of the parotid gland.
Design: The consultation files of one of the authors were reviewed from 2009 to present for salivary gland lesions having the previously described histologic features of keratocystoma. These tumors were composed of multicystic spaces arranged in a non-lobular architecture and lined by stratified squamous epithelium. The squamous epithelium must be lacking atypia and have an absent or discontinous, attenuated granular layer.
Results: Three cases of keratocystoma were identified. Clinically, all patients were female with an age range of 40-57 (mean: 49 years). The parotid gland was involved in all cases (right: 2 cases, left: 1 case). Grossly, the lesions ranged from 1.7-3.0 cm (mean: 2.5 cm) in size and were described as cystic masses containing soft, tan material. Histologically, the cases were composed of multiple cysts lined with keratinizing squamous epithelium with variable degrees of hyperkeratosis. In one case, no granular cell layer was present in any of the squamous epithelial lining, while in two cases a minority of the cysts were lined with squamous epithelium having an attenuated, discontinous granular cell layer. No atypia was identified within the squamous epithelium. In two of the cases, rare to occasional interspersed solid nests of squamous epithelium were present. The smallest lesion was composed of cystic structures only. In one case, parotid ductal epithelium was identified transitioning into the stratified squamous epithelial layer. All cases had a random, irregular distribution of the cysts and solid nests, with no lobular architecture apparent. The surrounding stroma appeared fibrotic with varying degrees of chronic inflammation present. In two cases, granulomatous inflammation was present, likely in response to keratinaceous debris. No skin appendages were identified within the adjacent stroma.
Conclusions: We describe three cases of keratocystoma, a rare parotid gland tumor that can mimic both benign lesions, such as dermoid cysts, and malignant lesions. To our knowledge, the current study presents the largest series of cases thus far described. Knowledge of this unusual, benign lesion is important, as it can be mistaken for malignant lesions such as metastatic squamous cell carcinoma or mucoepidermoid carcinoma.

1308 FANCD2 Expression Is Increased in HPV16+ Oropharyngeal Carcinomas

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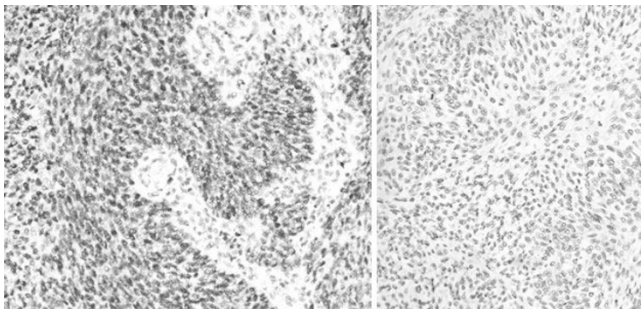
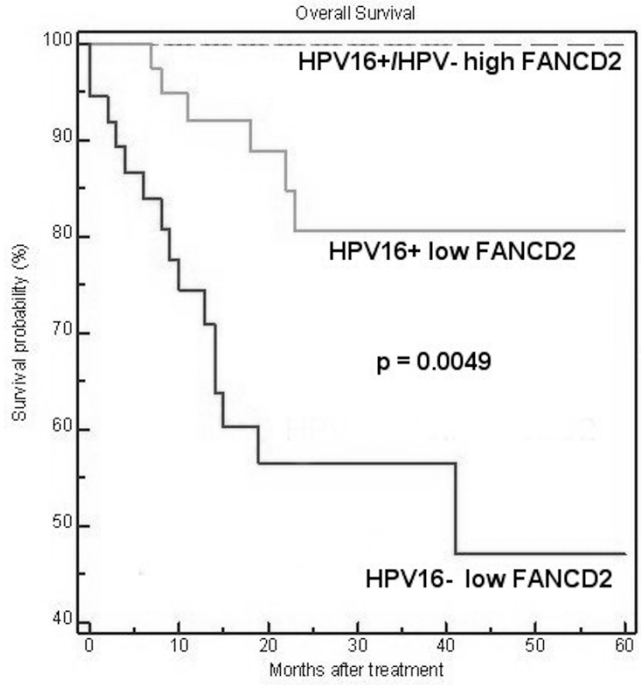
Background: The Fanconi Anemia pathway (FA or FANC/BRCA)is activated in response to chromosomal replicative stress. HPV16-E7 can activate the FA pathway, as E7 promotes unscheduled S-phase entry via cyclin deregulation. In turn, elevated FANC-D2 can limit HPV replication. Here, we investigate FANCD1/FANCD2 protein expression in oropharyngeal carcinoma (OPC) typed for HPV16 and HPV18.
Design: Archival specimens from 99 OPC patients were previously typed for transcriptionally active HPV16 and HPV18 by reverse transcription, and real time nested PCR using type specific E6 and E7 primers. FANCD1 and FANCD2 were assessed by IHC on whole slides; staining quantified by H-score (range 0 – 12). A representative image of positive (H=12) and negative (H=0) staining is shown.



Results: Transcriptionally active HPV16/18 was present in 64% of OPC (46% HPV16+, 7% HPV18+, and 7% HPV16/18+). High H-score was common for FANCD1 and did not correlate with HPV status. A trend is seen with high FANCD2 (H-score≥ 6/12) and HPV16 (p = 0.088, Fischer's exact test); no association was seen with HPV18. Time to overall survival (OS) was increased with high FANCD2 (p = 0.056); protective trends were seen for high FANCD2 and disease specific survival (p = 0.093) and disease progression (p = 0.395). Subgroup analysis by treatment (chemoradiation vs surgery +/- adjuvant therapy) suggests that this effect is not associated with improved response to chemoradiation. Stratifying for HPV16 and FANCD2 demonstrates that OPC patients with HPV16+ low FANCD2 (green line, Fig 2) have poorer OS than high FANCD2 OPC, regardless of HPV16 status (p = 0.0049).



Conclusions: Upregulation of FANCD2 is associated with HPV16+ OPC. As this protein inhibits HPV replication, it may be an important modulator of outcome for patients with HPV16-mediated OPC. Further studies are necessary.

1309 HPV L1 in Oropharyngeal Squamous Cell Carcinomas: Comparison and Correlation with p16, HPV ISH, and Outcome

DG Davis, DR Braxton, N Fatima, ST Momin, C Cynthia. Emory University, Atlanta, GA.

Background: The importance of human papilloma virus (HPV) in the development of oropharyngeal squamous cell carcinoma (OSCC) has been increasingly recognized. Numerous studies have demonstrated the improved prognostic significance of HPV positivity in OSCC. HPV L1 immunohistochemistry (IHC) uses a monoclonal antibody specific for the L1 viral capsid protein produced in the late phase of viral replication. The purpose of this study is to evaluate the effectiveness of HPV L1 IHC for the diagnosis of HPV in OSCC and to compare it to p16 IHC and HPV in-situ hybridization (ISH). HPV L1 was also correlated with overall survival.

Design: 74 cases of OSCC (52 surgical specimens, 22 fine needle aspirations [FNA] of cervical lymph node metasteses) were identified; HPV L1 (Lifespan Biosciences) IHC, p16 IHC, and HPV ISH were performed on 5 micron, formalin-fixed, paraffin-embedded sections. HPV L1 and p16 exhibited nuclear and cytoplasmic staining, while HPV ISH staining was noted in nuclei in a dot-like pattern. HPV ISH was used as the gold standard.

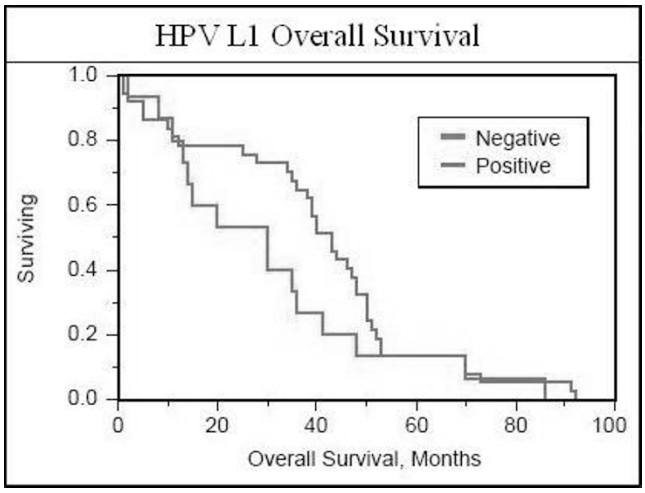
Patient Demographics

Age - Mean	58
Age - Range	28-80
Male/Female Ratio	4.7
Average Tumor Grade	2
Average Clinical Stage	4

Results: 52 (75%) of the cases (37 surgical specimens [79%] and 15 FNA's [74%]) stained positive for HPV L1 with an overall accuracy of 61%. Chi squared analyses revealed no significant correlation between HPV L1 and HPV ISH, and demonstrated a strong correlation (p<0.0005) between p16 IHC and HPV ISH. No significant difference in overall survival was seen in HPV L1 positive and negative OSCC's (p=0.1).

	HPV ISH Positive	HPV ISH Negative	
HPV L1 Positive	36	16	PPV - 69%
HPV L1 Negative	11	6	NPV - 38%
	Sensitivity - 77%	Specificity - 27%	Accuracy - 61%

	HPV ISH Positive	HPV ISH Negative	
p16 Positive	39	8	PPV - 83%
p16 Negative	13	14	NPV - 52%
	Sensitivity - 75%	Specificity - 64%	Accuracy - 72%



Conclusions: The majority of OSCC's studied were of advanced clinical stage and expressed HPV L1 primarily in the dysplastic mucosa adjacent to the invasive carcinoma. Our study demonstrated that HPV L1 IHC is a sensitive, but not specific, marker of HPV and is unlikely to be a useful adjunct to p16 IHC or HPV ISH in the evaluation of OSCC.

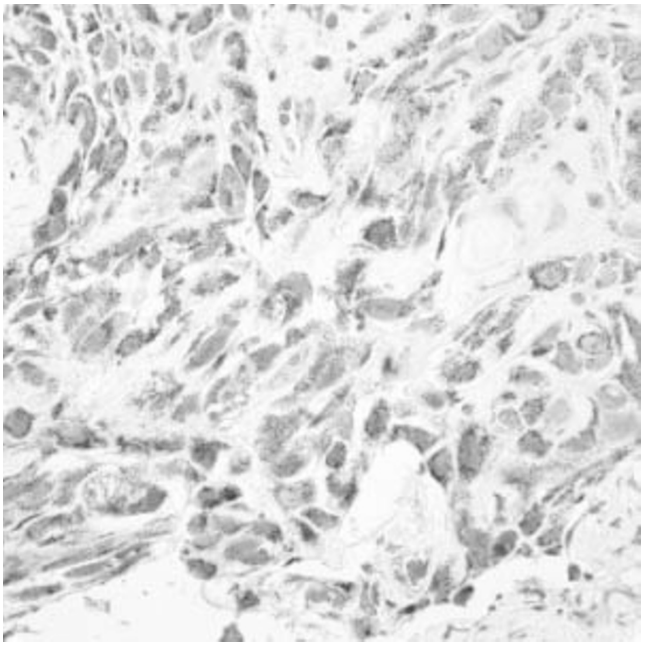
1310 Sirtuin-3 Immunohistochemical Expression in Oropharyngeal Squamous Cell Carcinoma: Correlation with Outcome

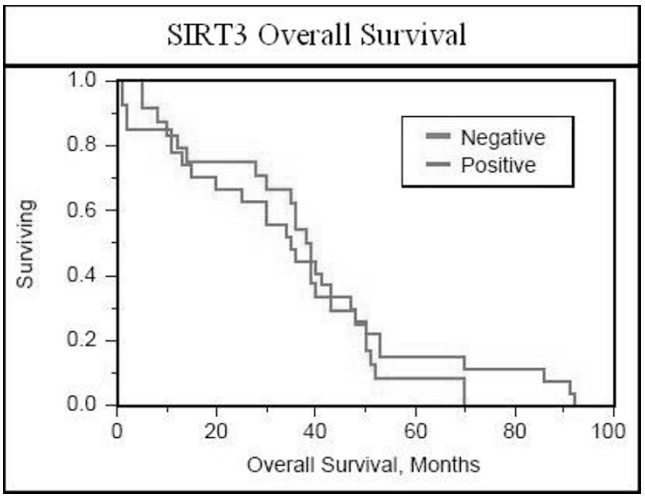
DG Davis, DR Braxton, N Fatima, BC Willis, MT Siddiqui, C Cohen. Emory University, Atlanta, GA.

Background: Recent studies reveal the importance of the sirtuin family of proteins. They function as tumor suppressors via their ability to reprogram mitochondrial metabolism during nutrient stress and through control of reactive oxygen species. A specific mitochondrial sirtuin, sirtuin-3 (SIRT3), has been investigated in oropharyngeal squamous cell carcinoma (OSCC) and is found to be up-regulated relative to benign squamous mucosa. We studied SIRT3 in relation to overall survival in OSCC.

Design: 69 OSCC's (47 surgical specimens and 22 FNA's of cervical nodes with metasteses) were identified. SIRT-3 immunohistochemistry was performed on 5µm, formalin-fixed, paraffin-embedded tissue sections. Cytoplasmic staining of SIRT3 was assessed as 0-3+ intensity (relative to controls of benign squamous mucosa) and the percentage of positive cells was recorded. Positive SIRT3 expression was correlated with overall survival.

Results: 36 (61%) of 69 total cases (30 surgical and 6 cytology specimens), stained positively for SIRT3 with a range of 1-3+ intensity. Positive staining was observed predominantly in poorly differentiated regions of invasive carcinoma. Minimal to no staining was seen in normal controls of oropharyngeal squamous mucosa and in normal mucosa adjacent to carcinoma. Positive SIRT3 IHC did not significantly affect overall survival.





Conclusions: SIRT3 expression did not affect overall survival. However, over-expression of SIRT3 in the majority of the OSCC's, particularly in more poorly differentiated tumors, confirms the findings of prior studies and reveals that SIRT3 alterations may be a factor in the molecular pathogenesis of OSCC. Recent findings of mutations in the active site of the enzyme in several cell lines isolated from OSCC raise the possibility that, while over-expressed, the enzyme may be functionally impaired. This may explain the observed correlation with SIRT3 expression in poorly differentiated foci of carcinoma.

1311 Whole Genome Expression Profiling of Epithelial- Myoepithelial Carcinoma of Salivary Glands

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Background: Diverse microarray/ sequencing technologies were used to characterise molecular changes in malignant epithelial cells in salivary neoplasms. Such gene expression studies to identify markers and targets in tumour cells are compromised by cellular heterogeneity of these tumours and by lack of appropriate counterparts representing normal salivary.

Design: 17 primary salivary epithelial-myoepithelial carcinomas and 6 normal salivary counterparts were microdisected from paraffin embedded tissue. Pools of RNA from highly enriched preparations of these cell types were subjected to expression profiling using a whole transcriptome shotgun sequencing experiment.

Results: Statistical correlation analysis of the resulting data allowed continuing with a comparative analysis of 6 normal samples versus 13 neoplastic samples, 4 neoplastic samples were excluded. Using strict /conservative criteria 220 differentially expressed transcripts were found, with 36% up- and 64% downregulated. The transcripts were annotated using NCBI Entrez Gene, and computational analyzed with the Ingenuity Pathway Analysis program. From these significantly changed expressions the analysis finds 26 cancer-related (ACAT1, ARPC1A, ATP5J, CANX, DDX39B, DDOST, EXT1, FGFR1, GOLPH3, MAGT1, MAPK8IP3, MAT2A, MMADHC, P4HB, PDK4, RHOA, SCARB2, SDHB, SDHD, SEC63, SSR1, SSR3, TM9SF2, TMED2, TRAM1, ZMPSTE24) and 16 transcripts which are related to mitochondrial dysfunction (ATP5A1, ATP5F1, ATP5J, COX7B, COX7C, MT-ND5, NCSTN, NDUFA4, NDUFA5, NDUFAB1, NDUFB11, NDUFV2, PDHA1, PRDX3, SDHB, SDHD), overlapping with 3 cancer-genes. These findings are well supported by existing literature about this cancer. From other strongly differentially expressed transcripts of microRNAs and certain genes the biological functions and their role in this cancer are still unknown.

Conclusions: These 220 differentially expressed genes and microRNAs provide for the first time, a sufficient large set to specifically define epithelial-myoepithelial carcinoma, with basis for the identification of novel and potentially important targets for diagnosis, prognosis and therapy in this cancer. Clinical pathological validation of a small number of genes is currently ongoing on tissue microarrays. These data will form the basis for understanding not only cell fate determination and cellular homeostasis in the normal salivary epithelium but also the contribution of different salivary epithelial cell types to the etiology and molecular pathology of salivary disease.

1312 Rab25 Expression as a Prognostic Marker in Oropharyngeal Squamous Cell Carcinoma

AE Foster, R Uppaluri, A Winkler, E Duncavage, J Lewis. Washington University, St. Louis, MO.

Background: Human papillomavirus (HPV)-related oropharyngeal squamous cell carcinoma (OSCC) has a quite favorable prognosis. However, a significant minority of patients suffer disease recurrence, particularly as distant metastases. Hence, discovering new genes that may contribute to tumor aggressiveness could potentially lead to more personalized treatment. We previously identified Rab25 as selectively mutated in patients with distant metastases in prior genomic studies, and other literature has suggested that it functions as a tumor suppressor.

Design: Immunohistochemistry was performed for Rab25 on a tissue microarray cohort of OSCC cases with known clinical follow-up and p16 status. p16 positivity was defined as≥50% immunostaining. Rab25 staining was assessed visually for percentage of tumor cells with cytoplasmic expression and stratified into quartiles. For analysis, this was dichotomized as >75% (4+) vs all others. Cases with Rab25 nuclear expression were read as positive or negative. Results were compared with clinicopathologic features and patient outcomes.

Results: 136 p16-positive OSCC were present on the array, of which 113 (83%) had >75% Rab25 expression. Low Rab25 expression (<75%) correlated significantly with development of distant metastases (p=0.02), with death from any cause (p=0.003), and had lower 5-year survival rates when compared with high Rab25 expression (55% vs 85%; p=0.01). However, there was no statistically significant difference when looking at death from disease (p=0.07). Low Rab25 expression was statistically significantly correlated with higher T-stage (p=0.02) and definitive vs surgical management (p = 0.006), but no significant difference was seen by Rab25 for N-stage, sex, race, or histological tumor type. Nuclear expression was seen in only 5 patients (3.7%), 1 (20%) of whom developed distant metastasis vs. 9 of 130 (6.9%) of the nuclear negative patients. However, the numbers are too small for meaningful analysis.

Conclusions: The majority of HPV-related OSCC cases show extensive tumor cell expression of Rab25. Reduced Rab25 expression correlates with poorer outcomes, suggesting it acts as a tumor suppressor, but this association is modest. Nuclear Rab25 expression is rare but may be a reflection of protein mutation correlating with tumor aggressiveness.

1313 Analysis and Significance of c-MET Expression in Adenoid Cystic Carcinoma of Salivary Gland

MD Fox, D Roberts, RS Weber, AK El-Naggar, D Bell. MD Anderson Cancer Center, Houston, TX.

Background: Increased copy number gain and amplification of c-MET, the cell surface receptor for hepatocyte growth factor (HGF), has been shown to enhance tumor growth and invasiveness and promote metastasis in certain tumor types. The relevance of its expression in adenoid cystic carcinoma (ACC) of salivary gland origin was first described in 2003 in a study published by Suzuki, et. al. Although their findings were novel, its small case number limited the impact of the study. In the present investigation, we evaluated the expression of c-MET in a large cohort of salivary ACCs and examined its clinicopathologic implications.

Design: Formalin-fixed, paraffin embedded tissue blocks of 199 cases of ACC of salivary gland origin, diagnosed at UTMDACC between 1986 and 2006, were retrospectively reviewed. Tissue microarray's from each case were constructed for immunohistochemical analysis of anti-c-Met antibodies. The results were independently evaluated by two pathologists.

Results: Expression of c-MET protein was seen in approximately half of the cases that were evaluated. There was no correlation between c-MET expression and overall patient survival at three and five years. Additionally, c-MET expression was not associated with any particular histologic type of ACC (cribriform, tubular, solid) and failed to demonstrate a relationship with tumor invasiveness as was previously considered.

Conclusions: Our findings contradict the purposed theory that c-MET expression, in ACC of the salivary gland, imparts a more aggressive clinical outcome. There appears to be no relevance for targeted therapy against c-MET protein in this sub-group of salivary gland tumors.

1314 Targeted Next-Generation Sequencing of Salivary Gland High-Grade Neuroendocrine Carcinomas

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Background: High-grade neuroendocrine carcinoma (NEC) of the salivary gland is a rare aggressive malignancy that usually arises in the parotid, has a male predominance, and a peak incidence in the 6th-7th decade of life. The majority express cytokeratin 20

(CK20) and are referred to as "Merkel cell type" because CK20 is also expressed by histologically similar cutaneous high-grade neuroendocrine (Merkel cell) carcinomas (MCC). Up to 80% of MCCs harbor the Merkel cell polyomavirus (MCPyV), which is not found in high-grade salivary gland NECs. Whole exome sequencing of MCCs show MCPyV-negative, but not -positive, cases to consistently have nonsense mutations in RB1. Besides the absence of MPCyV, little is known about the genetic landscape of salivary gland high-grade NECs.

Design: 4 cases of primary salivary gland high-grade NEC were retrospectively selected from our archives after excluding the possibility of metastasis from a skin or other primary site. 1 ug of DNA, extracted from formalin-fixed, paraffin-embedded tissue blocks using standard methods, was used to construct Illumina paired-end libraries. These libraries were then captured using a panel of 40 genes that are known to be clinically actionable in cancer and sequenced using 101bp paired-end reads at 815x coverage. Sequence data was analyzed using our institution's existing analysis pipeline. Significant variants were determined by looking for recurrently mutated genes and comparing data to publically available databases of somatic mutations (COSMIC, TCGA). PROVEAN and SIFT software was used to predict function of the significant variants.

Results: Next generation sequencing of a targeted gene panel identified an average of 316 (range 256-341) single nucleotide variants (SNV) per case. 2.1% of these SNVs resulted in coding region changes not reported in dbSNP. 9 somatic mutations were identified in 5 genes, of which 8 were considered to alter structure or be deleterious in 4 genes by PROVEAN and SIFT software (Table1).

TUMOR	HISTOLOGIC CELL TYPE	CK20	NOTCH1	PIK3CA	PTEN	RB1	TP53
1	SMALL	+	missense	missense (neutral)	-	-	nonsense
2	SMALL	-	-	-	-	-	missense
3	SMALL	+	-	-	missense	-	-
4	LARGE	+	-	-	missense	splice site	missense nonsense

Conclusions: Identified somatic mutations are predominantly found within tumor suppressor genes (TP53, RB1, PTEN). 3 of 4 cases harbor nonsense or missense mutations in TP53, while no case harbors nonsense mutations in RB1. These data suggest salivary gland NECs are genetically distinct from MCPyV-negative MCCs which consistently harbor nonsense mutations in RB1.

1315 Risk Stratification Categories for Salivary Gland Tumor Fine Needle Aspirates

CC Griffith, F Schneider, RK Pai, U Duvvuri, RL Ferris, JT Johnson, RR Seethala. University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: Salivary fine needle aspirates (FNA) can be useful in detecting malignancy, but have limitations in providing specific diagnoses. Also, there is currently no risk stratification scheme for categorizing salivary FNA as in other organ sites. We review our salivary FNA to devise standard diagnostic categories that can provide risk stratification and help refine clinical management.

Design: We identified 794 salivary FNA (1999-2012) of which 303 primary salivary epithelial lesions with corresponding surgical follow-up within 6 months were available for review. Adequacy was defined as at least 4 high power (400x) fields with the majority of the field consisting of epithelial cells. Cases were placed into categories based on cytonuclear features (cellularity, stroma, cytoplasmic tinctorial quality, nuclear grade and mitoses). Resection specimens were reviewed. Both risk of malignancy and risk of high grade malignancy were calculated for each category.

Results: Initial results for 98 aspirates (1999-2005) show a surprising 39% unsatisfactory rate (11% of which were cyst contents). Risk stratification categories are shown in the table. Despite limited numbers, pleomorphic adenoma and Warthin tumor appear to be distinct and comprise low risk categories. Cellular low grade basaloid neoplasms appear to have a somewhat higher risk of malignancy (including adenoid cystic carcinoma in this study), specifically when the stromal elements are non-fibrillary. Both vacuole rich and pleomorphic oncocytoid aspirates have a considerable risk of malignancy (mucoepidermoid carcinoma, acinic cell carcinoma, adenocarcinoma, salivary duct carcinoma) and are often high grade.

Rate of Malignancy by Cytologic Category

Category	N	Malignancy on F/U	HG Malignancy on F/U
Non-Neoplastic	7	0	0
Cyst Contents Only	10	2 (20%)	0
Rare Cells with High Cytologic Atypia	1	1 (100%)	1 (100%)
Pleomorphic Adenoma	13	1 (7.7%)	1 (7.7%)
Cellular Basaloid Neoplasm, Monomorphic	13	2 (15.4%)	2 (15.4%)
Warthin Tumor	6	0	0
Cellular Oncocytoid Neoplasm, Monomorphic	8	1 (12.5%)	0
Oncocytoid Neoplasm, Vacuole Rich	7	5 (71.4%)	3 (42.9%)
Oncocytoid Neoplasm, Pleomorphic	5	5 (100%)	5 (100%)

Conclusions: Categorization of salivary FNA into morphologic categories can provide a risk stratification scheme that follows the paradigm utilized in other organ sites and may allow more informed clinical management decisions. Inadequacy rates are high, and aspirates with 'cyst contents only' should not automatically be regarded as benign.

1316 Salivary Duct Carcinoma: Prevalence of Actionable Genetic Alterations and Re-Assessment of Conventional Clinicopathologic Prognosticators

CC Griffith, LDR Thompson, A Assaad, BM Purgina, C Lai, JE Bauman, I Weinreb, RR Seethala, SI Chiosea. University of Pittsburgh Medical Center, Pittsburgh, PA; Southern California Permanente Medical Group, Woodland Hills, CA; Virginia Mason Medical Center, Seattle, WA; University of Ottawa, Ottowa, ON, Canada; University Health Network, Toronto, ON, Canada.

Background: We characterized a large cohort of salivary duct carcinomas (SDC) by conventional clinicopathologic parameters and genetic alterations. Specifically, we assessed the potential phosphoinositide 3-kinase (PI3K) pathway activation due to p110α catalytic subunit of phosphoinositide 3-kinase (*PIK3CA*) mutation/amplification, *AKT1* mutations, or loss of phosphatase and tensin homolog (*PTEN*). Androgen receptor (AR) expression, *HER2* amplification, and the relationship between *PLAG1* and *HMGA2* rearrangements and *PIK3CA* mutations were evaluated.

Design: Clinicopathologic parameters (e.g., tumor size, TNM stage, precursor lesions) of 171 cases of SDC diagnosed in 5 centers (1956-2013) were correlated with overall and disease-free survival. Potential PI3K pathway activation was determined by testing for *PIK3CA* mutations (Sanger sequencing or SNaPshot PCR) and *AKT1* (Sanger sequencing) mutations, *PIK3CA* amplification (FISH) and/or loss of *PTEN* (FISH). *PLAG1* and *HMGA2* status and *HER2* amplification were also tested by FISH. AR status was determined by immunohistochemistry.

Results: AR was positive in 82% (83/101). *HER2* was amplified in 43% (10/23). *PIK3CA* mutations were detected in 32% (14/58): p.E545K (n=4), p.E542K (n=3), and p.H1047R (n=7). *PIK3CA* amplification was not detected (0/25). *PTEN* loss was found in 36% (9/25): homozygous deletion (n=3), chromosome 10 monosomy (n=4), and hemizygous deletion (n=2). No mutations were detected in *AKT1* (0/15). Overall, 36.2% (21/58) SDCs showed changes associated with PI3K pathway activation. Currently, we are exploring the relationship between *PIK3CA* mutations and previously reported *PLAG1* and *HMGA2* status (rearranged in 49%, 18/37).

Conclusions: AR expression, *HER2* amplification, and potential PI3K pathway activation are common targetable molecular alterations in SDC. Relationships among clinicopathologic and genetic events may uncover the sequence of molecular changes involved in SDC pathogenesis, elucidate a rational approach to personalized molecular testing, and suggest additional therapeutic options.

1317 Detection of Human Papilloma Virus in Non-Neoplastic Tissue of Patients with Primary Oropharyngeal Carcinomas: Does a Field Effect Exist?

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Background: The presence of a field effect in head and neck cancer has important clinical significance. Little information exists about this phenomenon in patients with human papillomavirus oropharyngeal squamous cell carcinoma (HPV-OPSCC). The aim of this study is to evaluate for HPV status in non-neoplastic tissue from patients with HPV-OPSCC.

Design: Ipsilateral and contralateral tonsillar tissue from patients with HPV-OPSCC was screened to identify blocks with non-tumor, non-dysplastic tonsillar epithelium. Control H&E slides were carefully evaluated for focal neoplasia. DNA was extracted and assessed for amplification of the L1 region of the HPV genome by real time PCR, using β-actin amplification as a control for DNA quality. HPV genotype was determined by melting curve analysis. Immunohistochemistry (IHC) for p16 protein was performed. Follow-up was collected form clinical charts.

Results: Specimens included 25 ipsilateral and 5 contralateral normal tonsillar sections from 25 patients. Tumor foci were identified on the control H&E in 6 cases (3 in situ and 3 invasive carcinoma). Of the remaining ipsilateral uninvolved tonsils, 74% (14/19) were negative for HPV while 26% (5/19) were positive for HPV 16. All contralateral tonsils were negative for HPV. β-actin amplification was adequate in all cases. Immunohistochemical studies for p16 stain demonstrated strong positivity in neoplastic cells, and patchy weak p16 positivity in adjacent normal epithelium and contralateral tonsils. There was no correlation between the HPV status and p16 staining in histologically normal epithelium. No recurrence was reported after a mean follow-up of 14.7 months.

Conclusions: HPV DNA was not detected in non-neoplastic tissue in the majority of our HPV-OPSCC cases (76% of adjacent normal tissue; 100% of contralateral tonsils). These findings suggest that HPV in patients with HPV-OPSCC does not generally establish widespread persistent infection in non-neoplastic tonsilar tissue. Thus, field effect is less of a concern for HPV-OPSCC than in smoking-related keratinizing oral squamous cell carcinoma.

1318 EWSR1 Rearrangement Is Diagnostically Useful to Differentiate Clear Cell Lesions of the Head and Neck

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Background: Multiple tumor types involving the head and neck (H&N) may contain clear cells and other overlapping histologic features, leading to diagnostic difficulties, particularly in small biopsies. EWSR1 rearrangements have been recently observed in two types of H&N tumors composed predominantly of clear cells (CC): hyalinizing clear cell carcinoma (HCCC) and odontogenic clear cell carcinoma (OCCC). Thus, this chromosomal alteration is an attractive diagnostic tool. Herein, we evaluated the presence of EWSR1 rearrangement in a variety of clear cell lesions of the H&N.

Design: Institutional pathology records were searched for H&N tumors using the terms "CC", "CC features", and "CC rich". All identified cases were reviewed and

subjected to fluorescence in situ hybridization (FISH) on paraffin sections using a commercially available EWSR1 dual-color break-apart probe (Abbott, Des Plains, Il) localized to chromosome 22 band q12. FISH results were scored blindly from morphologic diagnosis.

Results: Twenty eight cases with prominent CC component were retrieved, comprising 18 biopsies and 10 resections. Diagnoses included 10 HCCC (9 primary and 1 lung metastasis); 2 OCCC; 4 mucoepidermoid carcinoma; 3 poorly differentiated carcinoma NOS; 1 adenocarcinoma NOS; 1 CC oncocytic hyperplasia; 1 myoepithelial carcinoma; 1 squamous cell carcinoma; and 5 metastatic renal cell carcinoma. FISH was technically adequate in 24 cases. EWSR1 rearrangements were detected in 46% (11/24). Positive cases included 89 % (8/9) of HCCC and 100% (2/2) of OCCC. A case originally diagnosed as squamous cell carcinoma with CC features showed EWSR1 rearrangements and was reclassified as HCCC. All other tumors were negative for rearrangement.

Conclusions: FISH testing using dual color EWSR1 probe is a useful marker to distinguish HCCC and OCCC from other CC lesions of the head and neck, both in primary and metastatic lesions. Moreover, our findings validate the practicality of this approach in the diagnosis of small biopsies. The application of this approach will lead to more accurate classification of tumors with overlapping histologic features and improved prognostication and management decisions.

1319 Immunohistochemistry for β-Catenin in Fibro-Osseous Lesions of the Craniofacial Skeleton

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Background: The canonical Wnt/β-catenin pathway is involved in the formation of the craniofacial skeleton and head and neck tissues. Aberrant nuclear localization of β-catenin was recently described in a subset of odontogenic tumors suggesting analogous growth promoting mechanisms to those of desmoid fibromatosis and colorectal carcinoma. Fibro-osseous lesions of the craniofacial skeleton are a group of neoplastic, self-limited, mesenchymal proliferations in which β-catenin status is unknown. This study aimed to characterize the prevalence of aberrant nuclear localization of b-catenin in a large series of fibro-osseous lesions of the craniofacial bones.

Design: 172 fibro-osseous tumors were identified from routine pathology and consultation files of the authors. The diagnoses were confirmed by review of histologic, radiographic and clinical variables. Immunostaining was performed on formalin-fixed paraffin-embedded representative sections using the BD Biosciences (clone 14B) β-catenin antibody (1:400). Slides were scored for nuclear staining, independently by AEH and RJ, blinded to the diagnoses, with consensus reached on all discrepant results.

Results: The series consisted of 172 tumors from 160 patients (45 male, 115 female; average age 32, range 2-69). No patients had documented familial adenomatous polyposis syndrome. Nuclear β-catenin immunostaining was detected in 34 (20%) tumors overall with no correlation between age, gender, or tissue decalcification and nuclear positivity (p=0.2, 0.17, 0.12, respectively). Distribution of staining by tumor type is shown in Table 1. *Absent* nuclear β-catenin in fibrous dysplasia was the only diagnostically significant finding (p=0.0034).

Table 1. Immunohistochemistry for β-catenin in fibro-osseous lesions.

Diagnosis	n	Nuclear staining
Fibrous dysplasia	43	2 (5%)
Ossifying fibroma	32	9 (28%)
Cemento-osseous dysplasia	28	9 (32%)
Desmoplastic fibroma	7	4 (57%)
Juvenile ossifying fibroma	4	2 (50%)
Fibro-osseous lesion NOS	52	8 (15%)
Other	6	0 (0%)

Conclusions: Nuclear staining for β-catenin is relatively common among fibro-osseous lesions of the craniofacial skeleton implicating the Wnt/β-catenin pathway in the pathogenesis of a subset of these lesions. With the exception of fibrous dysplasia, the individual categories of fibro-osseous lesions show similar frequency of nuclear β-catenin positivity. Thus, the finding of nuclear β-catenin has limited utility in discriminating among the other entities. The molecular mechanisms underlying nuclear β-catenin accumulation in the positive tumors is under investigation.

1320 Oral Plasmacytoses and Head and Neck Plasmacytoma: A Morphologic, Immunophenotypic and Molecular Comparison

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Background: Dense plasma cell proliferation, often associated with significant morphologic atypia, can be seen in oral mucosa in various clinical conditions. Distinction between reactive plasma cell-rich lesions (PCL) and plasma cell neoplasms (PCN) may require further workup.

Design: Cases of oral PCL with additional workup performed between 2008-2013 were identified and compared with head and neck plasmacytomas from our archives. Clinical information, histologic, immunohistochemical (IHC) and molecular findings were reviewed. Presence and extent of acute inflammation, admixed lymphocytes, plasmacytic bi-, tri or multinucleation, anisocytosis, hyperchromasia, exocytosis, mitoses, conspicuous nucleoli, Russell and Dutcher bodies was semi-quantitatively assessed.

Results: 15 PCL samples (7 gingiva, 3 buccal, 2 hard palate, 1 tongue, 2 periodontal tissue) from 14 patients, 5 male and 9 female, mean age 60 (range 46-83) and 7 PCN samples (2 laryngeal, 3 oral, 1 sinonasal, 1 nasopharyngeal) from 6 patients, 5 male and 1 female, mean age 58.5 (range 39-82) were reviewed. 6 PCL patients had history of multiple myeloma, 4 of SCC, and 1 of lichen planus. 11 had single and 3 had multiple lesions. Clinical findings included leukoplakia, erythroplakia, oral lichen planus, verrucoid lesions, osteonecrosis, and periodontal cysts. Squamous mucosa was hyperplastic in 9 and dysplastic in 3. All PCL had Plasma cells (PCs) with variable degree of atypia, anisocytosis (100%), hyperchromasia (53%), binucleation (93%), Russell (87%) and Dutcher (33%) bodies but none showed prominent nucleoli, tri- and multinucleation, mitoses;the latter were seen in 57%, 71%, 43%, and 43% of PCN, respectively. Admixed lymphocytes, Russell bodies, exocytosis and acute inflammation were less common in PCN. PCs were polytypic in PCL by IHC (14/14), ISH (2/2) or PCR (3/3) and clonal in PCN by IHC (6/6), ISH (1/1) or PCR (1/1). CD56, BCL1 and CD43 were expressed in PCs in 1/6, 1/6, 1/4 of PCN and in 1/8, 0/5, 4/6 of PCL, respectively.

Conclusions: Oral PCLs are commonly associated with acute inflammation, extensive exocytosis and admixed lymphocytes. Tri- or multinucleation, mitosis and conspicuous nucleoli in PCs should prompt further workup. Dutcher bodies are usually present in neoplastic PCs but we found them in a significant number of PCL cases. CD43 expression in reactive PCs is common. These findings may help in assessing the need for and interpretation of workup of oral plasma cell infiltrates.

1321 Correlation of Lymphatic Vessel Density, Tumor Staging and Nodal Metastases in Patients with Advanced Laryngeal Cancer

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Background: The data regarding the significance of lymphatic vessel density (LVD) in advanced head and neck cancer is inconsistent. The goal of our study was to examine the relationship between LVD, tumor staging, and lymph node status in advanced laryngeal squamous cell carcinoma (SCC).

Design: A total of 55 laryngectomy specimens from previously untreated patients with advanced laryngeal SCC were included. Selected blocks from formalin-fixed paraffin embedded specimens were sectioned and stained with hematoxylin-eosin and immunostained with D2-40, specific marker for lymphatic endothelial cells. Tumor grade, tumor margin(infiltrating vs pushing), and tumor necrosis (<5%absent, >10% present) were evaluated. The intratumoral and peritumoral LVD was determined in tumor vessel "hot spots" using light microscope (20 x magnifications) by four observers. The mean vessel counts in 3 hot spots per section was recorded.

Results: Table 1. shows histologic parameters and LVD in pathologic stages 3 and 4 (T3 and T4) laryngeal SCC. In these patients the mean peritumoral LVD was 6.0 (T3-5.9 and T4-6.1), while the mean intratumoral LVD was 5.2 (T3-4.8 and T4-5.6). The Spearman's rho correlations showed a significant relationship between the presence of peritumoral (p=0.018)/ intratumoral LVD (p=0.045) and pathologic tumor stage. There was no significant correlation between peritumoral (p=0.326) and intratumoral LVD (p=0.875) and N (nodal) staging.

Table1 Histologic parameters and LVD in patients with advanced SCC

Category	Intratumoral LVD (Mean)	Peritumoral LVD (Mean)
Stage		
T3 (n=26)	4.8	5.9
T4 (n=29)	5.6	6.1
Tumor necrosis		
Present (n=38)	4.3	6.4
Absent (n=17)	6.3	10.5
Nodal stage		
N0 (n=21)	6.4	5.4
N+ (n=34)	5.0	5.7
Margins		
Infiltrative (n=40)	5.7	6.2
Pushing (n=15)	6.6	3.9

Conclusions: In this patient population, development of intra and peritumoral lymphatics in laryngeal squamous cell carcinoma is associated with tumor stage, but not with nodal status. Detecting tumoral lymphatic proliferation is another step in understanding tumor biology. Targeting of lymphangiogenesis may be of therapeutic benefit in selected group of patients with laryngeal SCC. Furthers studies, including evaluation of LVD in non-advanced laryngeal carcinoma, are needed to validate these findings.

1322 *PLAG1* and *HMGA2* Abnormalities in Differential Diagnosis of Carcinoma Ex-Pleomorphic Adenoma and *De-Novo* Counterparts

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Background: Carcinoma ex-pleomorphic adenoma (CA ex-PA) is a rare malignant salivary gland tumor that arises in association with pleomorphic adenoma (PA). Both PA and CA ex-PA have a broad spectrum of histologic features and distinction from its histologic mimics may be difficult based on morphology alone. *PLAG1* and *HMGA2* gene rearrangements and amplifications are the most common genetic events in both PA and CA ex-PA; however, the utility of *PLAG1* and *HMGA2* abnormalities as adjunct molecular tests in the diagnosis of salivary gland tumors has not been well established.

Design: FISH analysis for *PLAG1* and *HMGA2* was performed on 21 CA ex-PA (9 myoepithelial carcinomas (MECA), 10 Salivary duct carcinomas (SDC), 1 mucoepidermoid carcinoma, and 1 mixed MECA-adenocarcinoma NOS), 18 *de novo* carcinomas (11 MECA and 7 SDC), 16 PAs and 11 PA-histologic mimics (2 epithelial-myoepithelial carcinoma (EMC), 2 basal cell adenocarcinoma (BACA), 7 polymorphous low grade adenocarcinomas (PLGA)).

Results: All except 3 cases (86%) of CA ex-PA were positive for *PLAG1* or *HMGA2* rearrangements/amplifications. The three negative CA ex-PA were of SDC subtype and they showed a clear-cut benign PA component. In contrast, 17 out of 18 (94%) de novo carcinomas lacked abnormalities in *PLAG1* or *HMGA2* genes (p<0.01). *PLAG1* or *HMGA2* rearrangements were identified in 7/9 (78%) hypocellular myxoid PAs and in 2/7 (29%) cellular PAs. Furthermore, all morphologic mimics of PA were negative for *PLAG1* or *HMGA2* rearrangements.

Conclusions: *PLAG1* and *HMGA2* rearrangements are the most common genetic events in CA ex-PA regardless of the histologic subtype. Unlike CA ex-PA, *de novo* SDC and MECA were negative for *PLAG1* and *HMGA2* abnormalities. Interestingly, rearrangements of *PLAG1/HMGA2* were identified in the majority of hypocellular PAs but only in a small subset of the cellular PAs. FISH ancillary test for *PLAG1* or *HMGA2* abnormalities can be used to distinguish between PA and its morphologic mimics.

1323 A Distinct MicroRNA Signature Reveals a Novel MiR-146a-5p – NF-kB Putative Tumorigenic Pathway in Olfactory Neuroblastomas

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Background: Olfactory neuroblastoma (ONB) is a rare malignant neuroectodermal tumor that arises in the superior portion of the nasal vault and can be locally invasive. MiRs are a recently identified class of non coding genes which play major roles in posttranscriptional gene regulation. Gene signatures of malignant cells show abnormal miR expression patterns when compared to normal tissue. Herein, we identify a specific gene signature for ONB, validate deregulated miRs, and postulate the significance of these miRs.

Design: Five cases of ONB and 3 samples of normal nerve tissue were selected from tissue archives. RNA was extracted and analyzed using Nanostring. RT-PCR was used for mRNA analysis and validation of miR expression.

Results: Comparison of ONB vs normal nerve tissue showed deregulation of 56 miRs. Of these, 36 were downregulated and 20 were upregulated. Of the upregulated miRs, 17 were at levels more than double that of normal tissue. Of the downregulated miRs, 8 were expressed two times less and 3 were expressed three times less than normal. RT-PCR was used to validate three downregulated miRs: miR-32-5p, miR-146a-5p, and miR-612. RT-PCR also showed increased levels of NF-kB.

Conclusions: Olfactory neuroblastoma tumors have a distinct miR signature. This signature could potentially be used as a diagnostic tool to differentiate ONBs from normal tissue in samples with limited tissue, as well as to identify ONBs in metastases with unknown primary cancers. In this case, the miR profile of ONB shows downregulation of miR-146, a well known tumor suppressor gene which downregulates NF-kB. NF-kB is a transcription factor which promotes transcription of oncogenes and is often dysregulated in cancer. Consistent with this, we found that downregulation of miR-146a-5p correlated with increased expression NF-kB. This suggests that there may be a tumorigenic pathway involving miR-146a-5p and NF-kB leading to the development of ONB. Additional studies to further characterize this potential pathway and the importance of the other deregulated miRs are ongoing.

1324 HER2 Expression Correlates with *HER2* Amplification in Salivary Duct Carcinoma: Using a Novel Scoring System to Determine Targeted Therapy

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Background: Salivary duct carcinoma (SDC) is an uncommon salivary gland malignancy that is frequently associated with a lethal clinical course. Targeted therapies used against SDC are rooted in its overexpression of androgen receptor (androgen deprivation) and amplification of *HER2* (trastuzumab). Although HER2 scoring systems currently exist for breast and gastroesophageal adenocarcinoma, no such system exists for SDC. This study puts forth a novel HER2 scoring system designed for SDC that reliably predicts *HER2* amplification.

Design: Retrospective clinical and histopathologic review of 32 cases of SDC seen at Mayo Clinic (1961-2007) was performed. Clinical data and outcome parameters were obtained from medical records. Surgical pathology archival material was re-examined and formalin-fixed paraffin-embedded material was further evaluated with IHC for androgen receptor (AR) and HER2 expression. AR IHC (nuclear) staining was classified as negative (<50% of tumor cells) or positive (≥50% of tumor cells). HER2 IHC (membranous) staining was classified as 1) none, weak (not crisp, faint) or strong (crisp, dark); and 2) <50% or ≥50% of tumor cells. HER2 scoring was classified as: 0 = none; 1+ = weak, <50%; 2+ = weak ≥50% or strong <50%; and 3+ = strong ≥50%. Fluorescence *in situ* hybridization (FISH) with PathVysion HER2 DNA probe set (Abbott Molecular) was performed on all SDC and normal specimens. Interpretation followed ASCO/CAP 2007 guidelines. Positive predictive value (PPV) and negative predictive value (NPV) were estimated along with 95% score confidence intervals.

Results: Median patient age was 58y (range 32-75) and 23 (72%) were male. All 32 cases were AR positive. HER2 IHC scoring showed: 0 (7 cases, 22%); 1+ (4,13%); 2+ (2, 6%); and 3+, (19, 59%). *HER2* FISH showed the following in chromosome 17: amplification (19, 59%), polysomy (6, 19%), monosomy (1, 3%), and normal (6, 19%). Cases with a HER2 score of 0 were either normal (6) or monosomy (1); HER2 score 1+ and 2+ showed polysomy (6, 100%); HER2 score of 3+ represented amplification (19, 100%). The PPV of a 3+ IHC score was 100% (95% CI=83.2-100) and NPV scores <3+ IHC was 100% (CI=77.2-100).

Conclusions: HER2 overexpression can reliably predict *HER2* amplification in SDC. HER2 score of 3+, defined as strong membranous staining in ≥50% of tumor cells, showed a PPV of 100% for *HER2* amplification. The NPV for HER2 scores of 0-2+ was also 100%. This novel SDC HER2 scoring system may provide a cost-efficient manner to determine eligibility for targeted therapy such as trastuzumab.

1325 Regulation of Non-Coding RNAs by Human Papilloma Virus in the Pathogenesis of Head and Neck Squamous Cell Carcinoma

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Background: Most commonly known for its role in cervical cancer, human papillomavirus virus (HPV), primarily HPV16 and its oncoproteins E6 and E7, is also a major risk factor for oropharyngeal squamous cell carcinoma (OPSCC), a subset of head and neck cancers. Nonetheless, the molecular mechanism for the pathogenesis and progression of HPV-related oral cancer is poorly understood. MicroRNAs (miRNAs) are predicted to regulate up to 30% of protein-encoding genes, and are crucial for the pathological classification of tumors and elucidation of the pathogenesis of oral cancers. Long non-coding RNAs (lncRNAs) have been demonstrated to regulate transcription factors that are closely associated with regulation of self-renewal and differentiation, tumor suppressors, and protooncogenes. This study sought to determine how HPV causes malignant transformation of normal oral cells through regulation of miRNA and lncRNA upon initial expression of the E6 and E7 oncoproteins.

Design: Very early passage oral epithelial culture cells HOK and OKF4 were transfected with HPV16 expression plasmids, and analyzed with qPCR and RNAseq to determine lncRNA and EMT gene expression levels. Functional effects of let-7 and miR-296 were investigated through premiR transfection and forced expression of HPV16 E6/E7.

Results: Transfection with HPV expression plasmids induced the stem cell genes Oct4 and Nanog, elevated Vimentin and inhibited of E-cadherin levels to promote an epithelial-to-mesenchymal (EMT) phenotype. Through qPCR-based arrays and RNAseq, the miRNAs miR-296, let-7, and miR-197 were determined to have the biggest differences in expression between control cell lines and those that expressed HPV16 oncoproteins. Overexpression of let-7 and miR-296 decreased cell proliferation compared to control, which effects were partially reversed with forced expression of HPV16. Let-7 also decreased cell proliferation by sensitizing cells to cisplatin and increasing the Bax/BCL2 ratio, but forced expression of HPV16 reversed this ratio.

Conclusions: These results indicate that HPV16 can confer cancer stem cell characteristics to normal oral epithelial cells and induce the EMT phenotype by dysregulating miRs let-7 and miR-296. Further investigation of these miRs may elucidate the molecular mechanism of oncogenesis through HPV-regulated ncRNAs, and serve as both prognostic indicators and therapeutic targets for HPV-induced oropharyngeal cancers.

1326 Does p16 Status Matter in the Larynx? A Study of Overall Survival in p16 Positive Squamous Cell Carcinomas of the Larynx Regardless of HPV Status

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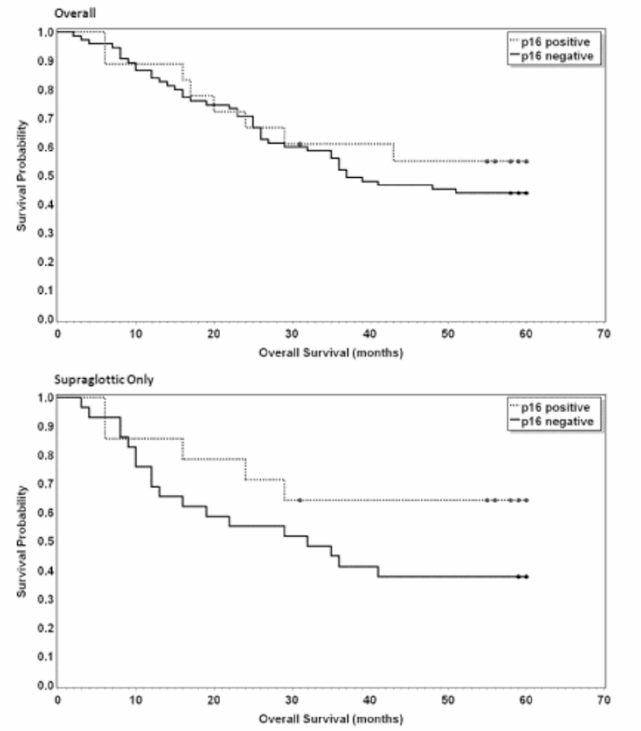
Background: HPV-driven squamous cell carcinomas (SqCCs) have shown marked anatomic specificity for the cervix and oropharynx. We previously investigated laryngeal squamous cell carcinomas, finding p16 positive tumors occur predominantly in the supraglottis and are rarely positive for HPV by in situ methods. It has been suggested that p16 is prognostically relevant regardless of HPV positivity. Herein, we analyze survival with regard to p16 status and laryngeal location.

Design: A computerized search yielded 150 laryngectomies removed for SqCC. Of these 103 were separated into primarily supraglottic (n=44), glottic (n=51), or subglottic (n=8) in origin. The rest were eliminated from the study. The tumors had known p16 immunohistochemistry and HPV 16, HPV high risk, and E6/E7 mRNA in situ studies. We gathered clinical data and performed correlation and Kaplan-Meier statistical analysis regarding tumor stage, gender, smoking status, and overall survival.

Results: Overall, 17.8% of tumors were positive for p16, although only 1 expressed both HPV-16 and E6/7 by in situ staining. The patients were 17.5% female and 82.5% male. 35% of patients were smokers, while 65% had no smoking history. The tumors were 16% (15 of 96) stage 1, 22% (21 of 96) stage 2, 19% (18 of 96) stage 3, and 44% (42 of 96) stage 4. Other than the previously described predilection for supraglottic sites, Chi-square analysis found no correlation between p16 positivity and gender, stage, or smoking history. p16 status also was unrelated to overall 5 and 10 year survival. As most p16 positive tumors were supraglottic, survival studies were additionally performed on this group separately. Although p16 positive tumors showed a trend toward better overall survival, there was no statistically significant difference (p=0.17).



Conclusions: As previously described, SqCCs of the larynx are rarely HPV-associated and show decreased rates of p16 positivity. Although supraglottic p16 positive tumors showed a positive trend, there is no definitive statistical evidence that p16 is associated with an improved outcome. Therefore, we feel that the use of p16 in the larynx, particularly in the absence of prototypic non-keratinizing morphology, is of minimal clinical utility.

1327 Proliferative Grading Predicts Outcome in Myoepithelial Carcinoma of Salivary Glands: A Clinicopathologic Study of 49 Cases

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Background: Myoepithelial carcinoma (MECA) is an underrecognized rare tumor with diverse clinical behavior. The histologic features of this tumor are not well characterized, much less its grading, which is controversial. The objective of this study is to provide a better characterization of MECA and its prognostic factors with the aim to establish a grading system for a better patient stratification.

Design: 49 cases of MECA were retrieved from the pathology file. The cases were subjected to a detailed histopathologic, immunohistochemical, and clinical analysis.

Results: Median patient age was 61 years (25-90) with a female/male ratio of 1/1. Tumors were located in parotid (71%), submandibular gland (14%), and minor salivary glands (12%) with an average size of 3.7 cm (0.9-9.5). Tumors were classified as de novo MECA in 22 cases (46%) and carcinoma ex pleomorphic adenoma (CAexPA) in 26 cases (54%). Histologically, all tumors showed invasive borders and most had multinodular growth pattern with a cellular periphery. In de novo MECAs, ductal formations were noted only in 4 cases and all had <10% ducts. Immunohistochemical stains were performed on 39 cases (77%), and each tumor was positive for keratin and at least one of the myoepithelial markers (S100, calponin, p63, and SMA). The other 11 cases showed the typical morphology of MECA. Tumor necrosis, mitotic count≥6/10HPFs, and severe pleomorphism were identified in 35%, 46% and 22%, respectively. PNI, VI, and positive margins were noted in 12%, 14% and 48%, respectively. The median FU was 38 months. 5 patients had LN metastasis at presentation, 9 developed local recurrences, and 11 had distant metastases (lung in 82%). Tumor size≥ 5 cm and the presence of PA component (CAexPA) correlated significantly with overall and distant recurrences ($P<0.05$). Tumor necrosis and/or mitoses of≥6 /10HPFs correlated significantly with overall and distant recurrences ($P=0.01$ and 0.0007, respectively). No distant recurrence was noted in patients with tumors without necrosis. The following did not correlate with recurrence (local or distant): atypical mitosis, severe pleomorphism, PNI, VI, positive LNs and positive margin.

Conclusions: Myoepithelial carcinoma is a relatively aggressive tumor that is associated with high rate of distant metastasis (24%). Compared to de novo MECA, CAexPA correlates with worse clinical behavior. A grading system based on the presence of tumor necrosis and/or increased mitotic activity should be used to identify high grade MECA and predict the clinical behavior for these tumors.

1328 Metabolic and Immunologic Characteristics of HPV+ oropharyngeal Cancers Indicating an Adaptive Mechanism to Increased Tumor Immunogenicity

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Background: Lymphopeithelial regions are predilection sites of HPV+ oropharyngeal squamous cell carcinomas (OPSCC), which show excellent response to radiotherapy. Solid tumors generally depend on aerobic glycolysis (Warburg effect), producing huge amounts of lactic acid, which promotes tumor progression and contributes to radioresistance. Additionally, lactic acid inhibits proliferation of activated T-cells, which also depend on aerobic glycolysis and lactate secretion. This study aimed to characterize the metabolic and immunological features of HPV+ and HPV- OPSCC to explain their distinct therapeutic behavior.

Design: A TMA based immunohistochemical analysis of 16 HPV+ and 17 HPV- OPSCC was performed for lymphocytic markers (CD3, CD4, CD8, CD20, Foxp3, Il17) and metabolic proteins (LDH, LDHb, Glut1, CD147, Cox5B). MRNA levels of Glut1, MCT1, LDHa and LDHb were assessed in OPSCC and in an HPV+ (UD-SCC-2) and HPV- (SCC4) OPSCC cell line. LDHa and LDHb levels were determined by western blot together with quantification of lactate secretion and assessment of proliferation in lactic acidosis using automated cell counting, XTT assay and real-time cell analysis.

Results: HPV+ OPSCC showed enhanced intratumoral lymphocytic infiltrates, particularly an increased CD8/CD4 ratio. In contrast, relative percentages of intratumoral Il17+ and Foxp3+ T-cells were decreased in HPV+ OPSCC. Expression of Glut1, CD147 and Cox5B was also increased in HPV+ OPSCC. Additionally, Cox5B expression correlated with the intratumoral CD8+ T-cell infiltrate. MRNA analyses confirmed a high metabolic activity of HPV+ tumor cells and indicated, based on an inverse relationship of LDHa (low in UD-SCC-2/high in SCC4) and LDHb (low in SCC4/ high in UD-SCC-2) together with decreased lactate secretion of UD-SCC-2, a lactate flux towards respiratory metabolism in HPV+ tumor cells. Consistent with this was the decreased number of intratumoral Il17+ T-cells in HPV+ OPSCC, which depend on the stimulating effects of lactic acid, and the capability of UD-SCC-2 to tolerate lactic acidosis demonstrating reduced inhibition of proliferation under lactic acid culture condition in several proliferation assays as compared to SCC4.

Conclusions: Our results indicate that HPV+ OPSCC have an enhanced aerobic metabolism, which might represent an adaptive mechanism to increased immunogenicity and could explain their preference for lymphoepthelial regions and good response to radiotherapy.

1329 Prevalence of Human Papillomavirus & P16ink4a in Multiple Synchronous or Metachronous Primary Squamous Cell Carcinomas of the Upper Aerodigestive Tract

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Background: The prevalence of HPV and p16Ink4a was investigated in patients with two or more primary HNSCCs.

Design: A retrospective study of patients with two or more HNSCCs was conducted in Royal Victoria Eye and Ear Hospital and St. James's Hospital, Ireland, between January 2000 and June 2012. p16 immunohistochemical staining was performed using purified mouse anti-human $p16^{INK4A}$ (BD Pharmingen™). The Qiagen DNA extraction kit was used to extract genomic DNA and it was tested for the presence of HPV using consensus SPF10 primers followed by gel electrophoresis. HPV genotyping was performed by TaqMan Real-Time PCR for HPV16, 18, 33 and 45.

Results: 72 (11.9%) out of 604 patients were identified with a second primary malignancy (SPM). Third primary malignancy prevalence rate was 3.0% (18/604) while a fourth primary malignancy rate of 0.5% (3/604) was established. Overall 57 males and 15 females between the ages of 36 to 78 (mean 58 +/- SD 9) were reviewed. The majority of SPM were located in the oral cavity (44%) and oropharynx (24%). Over 75% of these patients smoked and consumed alcohol. About one third of them (21pts) had HPV positive first primary tumour. Among them, nearly 50% (10pts) developed a HPV positive SPM. Majority of the patients were HPV-16 positive (12pts), 1 patient had HPV-18 and 2 patients had co-infection of HPV-16 and 18 in their index tumor. Interestingly, among the patients who were HPV negative in their first primary malignancy, approximately 35% a developed a HPV positive SPM. A low p16Ink4a positivity rate for both first primary (16.4%) and SPM (16.4%) was established. Patients who were HPV/p16 positive in both primary and SPM had a significantly higher 2-yr survival rate of 75% (95%CI 56.63, 93.38) compared to those that were HPV/p16 negative in both primary and SPM of 40% (95%CI 27.85, 52.15).

Conclusions: HNSCC patients that were both HPV positive and p16 positive develop a SPM and these patients have a better survival compared to patients who did not harbour HPV infections and were also p16 negative.

1330 Salivary Duct Carcinoma with Rhabdoid Features Is an Extremely Rare and Unique Subtype of Sarcomatoid Salivary Duct Carcinoma

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Background: Although salivary duct carcinoma (SDC) is a relatively common malignancy of the salivary glands, sarcomatoid variant of SDC (SSDC) is rare. The tumor cells showing rhabdoid features are extremely rare in SDC. Only one case of rhabdoid subtype was described as one of histological variations of SSDC.

Design: We extracted salivary duct carcinoma with rhabdoid features (SDCRF) cases from the pathology files of Shizuoka Cancer Center, Toyohashi Municipal Hospital, and Hyogo College of Medicine Hospital during 1995-2013. We examined them clinico-pathologically and immunohistochemically. Immunostain was performed for pan-cytokeratin (CK), epithelial membrane antigen (EMA), gross cystic disease fluid protein (GCDFP)-15, androgen receptor (AR), Her-2, epidermal growth factor receptor (EGFR), prostate-specific antigen (PSA), vimentin, p63, alpha-smooth muscle actin (ASMA), CK14, p53 and Ki-67.

Results: Five cases of SDCRF were selected. All cases were male with a mean of 59 years (range: 39-75 years), which included four parotid gland cases and one submandibular gland case. Two cases are alive with disease, whereas other two cases are dead. One case lost the follow-up. Histologically, four of five cases showed carcinoma ex pleomorphic adenoma, widely invasive type. The invasive portion showed diffuse proliferation of non-coherent large ovoid or polygonal carcinoma cells showing eosinophilic cytoplasm and eccentric nuclei. In situ lesions were also seen in pleomorphic adenoma areas. Immunohistochemically, such cells were positive for pan-CK, EMA, GCDFP-15 and AR in all cases, whereas they were negative for vimentin. Four cases showed Her-2 overexpression and one cases was positive for EGFR. Only one case was positive for PSA. Such rhabdoid cells were negative for myoepithelial markers, such as p63, ASMA and CK14. The nuclear accumulation of p53 protein was infrequently observed, whereas such carcinoma cells showed high labeling index of Ki-67 in invasive areas. Finally, we diagnosed the carcinoma component of these cases to be "SDCRF".

Conclusions: SDCRF is an extremely rare subtype of SSDC, and such a subtype indicates an aggressive clinical behavior, like conventional SDC. "The rhabdoid cells" in SDCRF should be distinguished from the plasmacytoid cells in other salivary gland tumors. The former are negative for myoepithelial markers.

1331 $p16^{INK4a}$ Immunohistochemical Expression Is Not a Surrogate Marker of HPV Presence in Laryngeal Squamous Carcinomas

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Background: $p16^{INK4a}$ overexpression has been proposed as a potential biomarker for HPV presence in a variety of squamuous cell carcinomas (SCC) including those of head and neck. The aim of this study was to determine the relantionship between $p16^{INK4a}$ expression and HPV presence in laryngeal (L)SCC.

Design: Patients with LSCC diagnosed at a single institution from 1992 to 1994 were restrospectively evaluated according to sample availability. Clinico-pathological features were retrieved from institutional records. Fifty-four LSCC were tested for HPV DNA by PCR using three different protocols based in MY09/11 and GP5/6 primers. $p16^{INK4a}$ expression was examined by immunohistochemistry (IHC) in 44 of the tumors. $p16^{INK4a}$ mRNA expression was analyzed through real-time quantitative reverse transcriptase-PCR in 34 cases. The results were correlated to $p16^{INK4a}$ mutation information previously available.

Results: All patients were male with ages of 61±16 years (mean ± SD). Ninety-one percent of tumors were at an advanced stage (49/54). All tumors were HPV-negative with all three detection methods in spite that all cases showed amplification of internal controls. Four out of 44 (9%) cases overexpressed $p16^{INK4a}$ by IHC. Two cases positive for $p16^{INK4a}$ immunostaining for which genetic information was available harbored p16 mutations (one missense and one frameshift). p16 mRNA expression was 0.7±0.49 (mean±DS) and ranged from 0.01 to 2.2 with a median of 0.49. Eight out of 9 mutated cases presented $p16^{INK4a}$ mRNA overexpression (above 0.49) whereas it was found only in 9 out of 25 non mutated cases (p=0.017, Fisher's exact test).

Conclusions: Our results show that HPV implication in LSCC in our setting is, at most, marginal. $p16^{INK4a}$ overexpression, both at protein and mRNA levels may reflect $p16^{INK4a}$ genetic alterations instead.

1332 Oral Preneoplastic Lesions: A Clinicopathological, Immunohistochemical and Molecular Approach

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Background: Most of the patients with multifocal diachronic or synchronic squamous cell carcinoma (SCC) of the oral mucosa have also multifocal preneoplastic lesions. Smoking and alcohol are important carcinogenic agents , but in a large number of cases the etiologic factor is unknown, as occurs in the proliferative verrucous leukoplakia. With the aim of investigating oncogenetic events in the preneoplastic oral lesions in different settings, several oncogenes were explored, as well as the HPV-association.

Design: Twenty patients with oral squamous cell carcinomas and preneoplastic lesions were included in the study. Ten patients met the proliferative verrucous leukoplakia condition. Clinicopathological data were recorded and lesions were histologically assessed. In all cases HPV analysis was carried-out by in situ hybridization (GenPoint HPV DNA Cocktail, Biotinylated, Dako) and immunohistochemistry for p16, p53, cyclin D1, and Ki 67 was performed. From 14 formalin-fixed paraffin embedded samples of 7 patients multiplex ligation-dependent probe amplification was performed with the SALSA MLPA P175 Tumour-Gain probemix (MRC-Holland).

Results: The patients were 14 females and 7 males with a median age of 65 (range 41-90). Seven patients were smokers, and 2 consumed alcohol. All SCC were negative for HPV. Increased CCND1 (11q13) and MYC (8q24) genes copy number was found in 83% and 50% of SCCs, respectively. Only 2 high-grade dysplasias showed increased CCND1 and MYC genes copy number, whereas low-grade dysplasias did not show any gene gain. Immunostain for p16 was negative in all tumors and dysplastic lesions. There was a positive correlation between the expression of p53, Ki67 and cyiclinD1 and the grade of dysplasia.

Conclusions: Multifocal preneoplastic and SCC in the oral cavity are usually not associated with HPV-infection. The oncogene gains are a late event in the development of SCC.

1333 Oncogenic HOXA10 Is Regulated by the Tumor Suppressor miR-494 in Oral Cancer

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Background: Squamous cell carcinoma (SCC) is one of the most common cancers of the oral cavity. MicroRNAs (miRs) are endogenous small non-coding RNAs and posttranscriptional regulators of gene expression in both normal and pathological processes. In our miRNA microarray analysis, miR-375 and miR-494 were identified as the two most underexpressed in human oral SCC versus controls. HOXA10, belonging to the homeobox family of developmental genes, is reported to be aberrantly expressed in several cancer types. A previous report showed up-regulation of HOXA10 in oral SCC-derived cell lines compared to human normal oral keratinocytes. HOXA10 is predicted as a candidate of both miR-375 and miR-494 target based on bioinformatics. This study aimed to analyze the interaction between miR-375/miR-494 and HOXA10 in oral SCC.

Design: HOXA10 expression was measured using qRT-PCR in oral SCC, normal tissues, and transfected cells normalized to GAPDH. Oral cancer cell lines SCC-25 and CAL 27 were transfected with miR-375-mimic, miR-494-mimic, and miR-mimic negative control, using Lipofectamine 2000. Proliferation of transfected cells were monitored using the trypan blue method. Mann-Whitney *U* test and Two-tailed Student's *t* test were used for tissues and *in* vitro experiments, respectively. The statistical analyses were performed using GraphPad Prism 5.0.

Results: Overexpression of HOXA10 was demonstrated in oral cancer tissues vs. normal tongue tissues (p=0.0048). HOXA10 was overexpressed in both early and advanced stages (p=0.052 and p=0.0046, respectively). Transient transfection of miR-494 in oral cancer cells reduced the expression of HOXA10 in SCC-25 and CAL 27 cells (p=0.0001 and p<0.0001, respectively) while miR-375 did not show any effect. Furthermore, both miR-494 and miR-375 reduced the proliferation of SCC-25 (p=0.0022 and p=0.0009, respectively) and CAL 27 (p<0.0001 and p=0.0038, respectively) cell lines.

Conclusions: This study suggests that, in oral cancer, overexpression of miR-494 could lead to reduce the oncogenic expression of HOXA10, however HOXA10 is not a target for miR-375. Additionally, miR-494 and miR-375 reduces the proliferation of oral cancer cells, reinforcing their role as tumor supressors miR in oral cancer.

1334 P16 Immunohistochemistry Versus HPV16 DNA Status in Predicting Survival in Locally Advanced Oropharyngeal Squamous Cell Carcinoma

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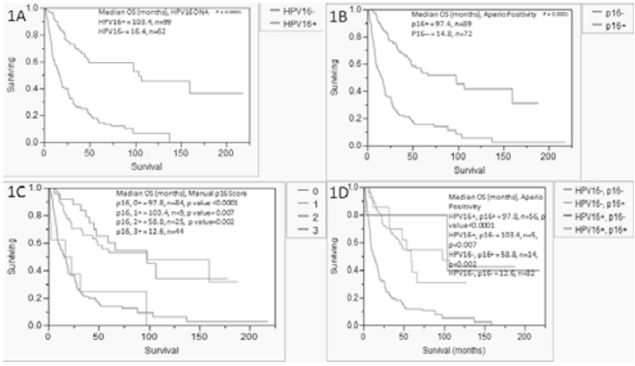
Background: HPV status is an important prognostic marker in oropharyngeal squamous cell carcinoma (OPSCC) with the majority caused by HPV16 type (80%). HPV positive status confers a significantly favorable prognosis. p16 immunohistochemistry (IHC) is increasingly used as a HPV surrogate marker. The prognostic significance of focal p16 positivity has yet to be elucidated. We investigated p16 IHC scored both manually and by digital image analysis and compared its prognostic significance vs. HPV16 DNA status in primary, locally advanced OPSCC.

Design: We retrospectively identified 165 cases of primary, locally advanced OPSCC (clinical stage III-IVB) diagnosed between 1992-2007 at University of Maryland Cancer Center with availabe paraffin-embedded tumor. 156 patients had both p16 IHC and HPV16 PCR testing done on E6/E7 oncogenes, 5 patients had indeterminate HPV16 PCR testing, 4 patients had no additional tissue for p16 testing. The p16 status was scored by Aperio positivity algorithm on representative tumor and manually scored by a blinded pathologist on a 0-3 scale (0=negative, 3=diffuse, strong). Median overall (OS) was defined as time from last followup to diagnosis. Frequency analysis was used to determine the cutoff for p16 positive/negative status.

Results: HPV16+ (n=98) did significantly better than HPV16- patients (n=62), median OS of 103.4 vs. 16.4 months (p<0.0001).



Aperio positivity analysis (5% cutoff) showed p16+ (n=72) had significantly better median OS than p16- patients (n=89): 97.4 vs. 14.8 months (p<0.0001). Manual scoring (2-3+ vs. 0-1+) of p16+ patients (n=69) vs. p16- patients (n=92) showed similar results: 97.8 vs. 16.5 months (p value <0.0001). Chi squared test of HPV16 vs. p16 manual or computerized scoring showed high correlation (p<0.0001), 19/156 cases were discordant. The discordant patients (HPV+, p16- or HPV-, p16+) still performed significantly better than HPV-, p16- patients.

Conclusions: P16 IHC is an alternative to HPV DNA based testing with similar prognostic results in locally advanced OPSCC. Weak focal p16 IHC was prognostically the same as p16- cases. Discordant HPV16, p16 patients may represent an intermediate risk group.

1335 Mutational Profiling of Ameloblastoma Identifies Common Gain-of-Function Mutations

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Background: Ameloblastoma is a locally destructive tumor of odontogenic epithelium that occurs in the jaw (maxilla and mandible) and often requires disfiguring wide local excision due to high rates of local recurrence and insensitivity to chemoradiation therapy. A comprehensive interrogation of the ameloblastoma genome has not been published primarily due to low tumor prevalence and exposure to decalcification which severely compromises nucleic acid integrity. Only recently have methods emerged to allow for deep sequencing from formalin-fixed paraffin-embedded (FFPE) archival tissue.

Design: RNA-seq was performed on archival FFPE blocks from two cases of ameloblastoma. Next, targeted next-generation sequencing of 9 samples of ameloblastoma, from 8 patients including the two investigated by RNA-seq, was performed using the Tru-Seq cancer panel (Illumina). Interesting findings were then confirmed as somatic via traditional Sanger sequencing of tumor-normal pairs in the 8 patients. The most significant molecular findings were then explored via Sanger sequencing in a multi-center cohort consisting of 37 additional cases. Finally, additional mechanistic studies were performed to query the functionality of select mutants.

Results: Preliminary RNA-seq analysis revealed several interesting mutations in the initial two cases, including mutations in the Hedgehog, Ras-Raf-MEK-ERK, and fibroblast growth factor pathways. These mutations were confirmed to be present and somatic in the initial larger test cohort of 9 cases. In addition, the genomic alterations were found to be present at high allele frequencies. Targeted sequencing of the larger multi-center validation cohort showed a majority of cases harbored at least one of the aforementioned mutations. Functional interrogation of one of the Hedgehog pathway mutants showed basal activation of the pathway, as well as resistance to various pharmaceutical pathway inhibitors.

Conclusions: Our study represents the first to profile the genomic mutational landscape of ameloblastoma. Not only were several canonical mutations identified that describe a majority of cases from multiple institutions, but the findings provide new mechanistic insights into the neoplastic process.

1336 Novel Karyotypic Abnormalities in Salivary Gland Tumors

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Background: Recurrent chromosomal aberrations have been well described in soft tissue tumors but limited information is available in solid tumors. Recently, a growing number of molecular studies have identified unique and recurrent genetic abnormalities in salivary gland tumors. We present novel and recurrent cytogenetic abnormalities in salivary gland tumors at our institution.

Design: Fresh tissue from tumors was obtained at the time of surgery and cultured in RMPI 1640. Chromosome analysis was performed on in situ cultured cells using Yale Molecular Cytogenetics Laboratory standardized procedures. Cultured cells were treated with colcemid to induce metaphase arrest and trypsin to digest chromosomal proteins. Wright's stain was performed for G-banding. Clonal abnormality was defined by similar numerical and structural chromosome rearrangements observed in at least three metaphases.

Results: Forty-seven primary salivary gland tumors (31 benign and 16 malignant) were successfully karyotyped between 4/2011 and 8/2013. Abnormal karyotypes were identified in 55% (27/47) of tumors: 19 benign (61%) and 8 (50%) malignant. In the pleomorphic adenomas with abnormalities, 33% (5/15) were 8q12 rearrangements and 20% (3/15) were 12q13-15 rearrangements. Novel karyotypic abnormalities were identified in 56% (15/27) of abnormal cases.

Figure 1

Diagnosis	# (%) of Cases	# (%) Abnormal	# Novel Abnormalities	Novel Abnormalities
All Cases	47 (100)	27 (55)	15 (56%)	
Malignant	16 (34)	7 (44)	7	
Acinic Cell Carcinoma	2 (4)	1 (50)	0	
Adenoid Cystic Carcinoma	3 (6)	2 (67)	2	t(3;13)(p24;q21)
				-Y
Basal Cell Adenocarcinoma	1 (2)	1 (100)	1	inv(8)(q12q24), idem,del(6)(q22q25)
Carcinoma Ex Pleomorphic Adenoma	1 (2)	0	0	
Epithelial Myoepithelial Carcinoma	2 (4)	1 (50)	1	+8
Mucoepidermoid Carcinoma, Low Grade	1(2)	1 (100)	1	+X, inv(2)(p22q26)
Myoepithelial Carcinoma	3 (6)	0	0	
Salivary Duct Carcinoma	2 (4)	1 (50)	1	66<3n-> complex numerical and structural rearrangements
Sebaceous Carcinoma	1 (2)	1 (100)	1	-Y, t(7;14)(q21;q22)[3]/t(1;8)(p10;p10), inv(11)(q14q13)[cp3]/t(11;22)(p11.2;q12)[2]
Benign	31(66)	19 (61)	8	
Basal Cell Adenoma	3(6)	1 (33)	1	der(1)t(1;4)(p13;q11),-4
Lymphoepithelial cyst	1 (2)	0	0	
Nodular Oncocytosis	1(2)	1 (100)	1	+7
Oncocytoma	1 (2)	0	0	
Pleomorphic Adenoma	21 (45)	15 (71)	6	ring chromosome
				t(20;21)(p11.2;q11.2)
				+1, add(1)(p22), der(1;6)(p10;p10),add(3)(q22),-5,add(5)(p13),del(13)(q12q22),add(22)(q13)
				-5q
				inv(5)
				add(5)(p13),del(9)(q31)
Warthin Tumor	4 (9)	0	0	

Conclusions: Multiple novel karyotypic abnormalities were identified in benign and malignant salivary gland tumors in addition to previously described recurrent abnormalities. The frequency with which karyotypic abnormalities are detected in salivary tumors paired with the continued identification of novel cytogenetic abnormalities supports karyotyping all salivary tumors. The identification of recurrent and novel chromosomal abnormalities will deepen our understanding of salivary tumorigenesis and may lead to the discovery of novel diagnostic, prognostic and predictive markers.

1337 Prognostic Significance of CD163 Positive Tumor Associated Macrophages in Oropharyngeal Squamous Cell Carcinoma

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Background: Macrophages are the most abundant cancer stromal cells of the host immune system, and it is known that tumor-associated macrophages (TAMs) are a major cellular component of human cancers, yet their impact on cancer biology is still unclear. CD163 positive TAMs have been shown to correlate with tumor progression and poor outcome in a variety of cancer types. However, their role in oropharyngeal squamous cell carcinomas (OPSCC), most of which are associated with human papillomavirus (HPV), has not been studied to date.

Design: CD163 immunohistochemistry was performed on a tissue microarray cohort of OPSCC cases with known clinical follow-up and HPV status (by RNA in situ hybridization [ISH]). Expression was assessed visually by one study pathologist, and was graded separately in intra- and peri-tumoral regions based on the extent of staining (1 = patchy, 2 = moderate, 3 = abundant) and intensity (1 = weak, 2 = moderate, 3 = strong). A combined intra- and peri-tumoral score of 2 or 3 was considered low, and a combined score of 4, 5 or 6 as high. Survival curves were estimated by the Kaplan-Meier method.

Results: A total of 183 patients, from 1996 to 2007**,** were studied, most of whom were men (88%) of Caucasian race (89%), and current or former smokers (67%). HPV RNA was positive in 81%. High (3+) intra- and peri-tumoral CD163 positivity was observed in 12% and 33% of the tumors, respectively. An overall combined high CD163 (4 or more) was seen in 55%. High CD163 was strongly associated with overall (p= 0.03) and disease free survival (p= 0.03). Among the HPV positive patients, high CD163 expression was even more strongly associated with survival, including overall (p= 0.02), disease specific (p= 0.02) and disease free survival (p= 0.02). There was also a correlation between high CD163 and patients receiving surgical treatment (p= 0.03) versus non-surgical, and with Caucasian race (p= 0.0002). However, no correlation with T or N-stage, co-morbidities, smoking, or drinking was found (p > 0.05).

Conclusions: Increased number of CD163 TAMs correlates strongly with patient survival in OPSCC, including among HPV positive patients. This is a novel finding and may suggest that macrophages are heterogeneously activated depending on the tumor type and organ specific microenvironment. In particular, it supports the concept that immune responses to HPV positive OPSCC are important in its pathobiology.

1338 Diagnostic Discrepancies in Mandatory Slide Review of outside Head and Neck Cases: An Institutional Experience

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Background: Each year in the U.S., medical error results in up to 98,000 unnecessary deaths, over a million injuries and substantial cost. Given that pathologic diagnoses are one source of medical malpractice, second review of material from patients seeking treatment at our institution has been mandatory. The few prior studies have shown that second opinion pathology review results in a clinically major diagnosis change in a significant minority of patients, ranging from 0.6% to 5.8%. In head and neck (H&N) pathology, specifically, reported rates of changed diagnoses have been higher. Our goal was to evaluate the diagnostic discrepancy rates in cases referred to our institution and to identify specific areas with more susceptibility to errors that may require subspecialty pathology expertise.

Design: Five hundred consecutive scanned H&N pathology reports (2009-2011) from non-directed consults were retrieved and compared for discrepancies between the outside and in-house diagnoses. All cases had been reviewed by at least one of three H&N pathology specialists at the time of referral. In our study, major discrepancies were defined as those resulting in a significant change in patient management and/or prognosis. Additional follow-up information, when available, was used for confirmation.

Results: Major discrepancy occurred in 29 (5.8%) cases. The majority (27/29, 93%) of cases were from private practice institutions. Informative follow-up material was available on 16/29 (55.1%) cases, among which the second opinion was supported in 13 (81.2%). Twenty-two (75.8%) of the major discrepant diagnoses were on biopsy material, most commonly involving the oral cavity. This was 9 of 29 (31%) major discrepancies but 9 of 100 (9%) of all oral cavity consults. Overall, dysplasia versus invasion was the most common (9/29, 31%) area of discrepancy and site wise, sinonasal cavity (21.7%) followed by nasopharynx (18.2%) had the most discrepant diagnoses. Of the major discordant diagnoses, 16 (55%) involved a change from benign to malignant, 2 had a change from malignant to benign (7%), and the remainder (38%) involved tumor classification.

Conclusions: Despite widely available resources and ancillary studies, H&N pathology is still a high-risk field, prone to erroneous diagnoses, and our study supports the importance of second opinion review by a specialty pathologist for the best care of patients. It may be helpful to better educate residents and fellows in specific areas (such as oral dysplasia) in order to prevent future incorrect diagnoses.

1339 *USP6* Rearrangements Are Not Identified in Giant Cell Reparative Granulomas of the Sinonasal Tract

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Background: Giant cell reparative granuloma (GCRG) is a benign lesion that many consider a solid variant of aneursymal bone cyst (ABC). Although primarily an intraosseous lesion, in the sinonasal tract GCRP can involve the soft tissues and present as an exophytic, polypoid mass. Recently, many ABCs have been shown to indeed be neoplastic and harbor recurrent translocations involving the ubiquitin protease USP6. Due to their morphologic similarities, we hypothesized that GCRP of the sinonasal tract will harbor this translocation as well.

Design: 13 cases of GCRG arising in the in the sinonasal tract were retrieved from the archives and clinical histories reviewed. Fluorescence *in situ* hybridization (FISH)

utilizing a commercially available dual-color break-apart probe targeting the *USP6* gene region (Empire Genomics, Buffalo, NY), was performed on full thickness sections of formalin-fixed, paraffin-embedded tissue. A case of nodular fasciitis (with a known *USP6* disruption) was used as positive control. Interphase nuclei were scored using a fluorescent scope and 150x oil immersion lens. 100 cells were scored as "intact" or "disrupted". A tumor was considered rearranged if greater than 10% of tumor cells were disrupted.

Results: Cases included tumors from 8 males and 5 females. 9 cases occurred in the maxillary sinuses, 3 cases presented as nasal masses, and 1 case occurred in the sphenoid sinus. 11 of the cases were primary resections, while 2 were of recurrences. By FISH analysis, 4 cases were not interpretable (likely due to prior decalcification procedure). No *USP6* rearrangements were detected in the remaining 9 cases, with intact signals seen in all cells including both the individual spindle cells and giant cell nuclei of each lesion.

Conclusions: Although *USP6* rearrangements have been shown to occur in most ABCs and occasional GCRGs, those lesions diagnosed as GCRC in the sinonasal tract do not appear to harbor rearrangements. These results suggest that even though morphologic similarities exist between sinonasal GCRGs and solid ABCs, the two lesions appear to be genetically distinct. Further studies will be needed to completely evaluate whether these proliferations are true neoplasms.

1340 Interobserver Variability in Assessing p16 Expression in Head and Neck Squamous Cell Carcinoma

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Background: p16 is widely used as a surrogate marker for human papillomavirus (HPV)-associated head and neck squamous cell carcinoma (HNSCC). Various cutoff values ranging from 35% to 75% have been proposed for a positive result for clinical practice and trials. We assess here the interobserver agreement in evaluating p16 expression in a HNSCC cohort of various sites.

Design: H&E and p16 slides of 196 HNSCC previously clinically tested for HPV status with p16 (mtm, Heidelberg, Germany) and in situ hybridization (HPV16/18 probe, Dako, Carpinteria) in our department were separately, independently evaluated by 3 observers with different experience in this assessment blinded to demographics, smoking status, original interpretation and tumor site. Presence of keratinization, percentage of p16 reaction ($p16^{\%}$) in tumor cells (1:<5%;2:5-25%;3:26-50%;4:51-75%;5>75%) and overall positive/negative/inconclusive interpretation of each observer without a preset cutoff was recorded. Discrepant $p16^{\%}$ and scores were resolved in a consensus meeting. Correlation of consensus p16 expression ($p16^{cons}$) with tumor site, keratinization, and previous result was evaluated.

Results: There were 62 oropharyngeal (OP), 60 oral (O), 20 overlapping (OOP), and 53 other primary sites SCC. Tumors were classified as keratinized (K, 71%), non-keratinized (NK, 21%) and ambiguous/mixed (8%). A total of 72 cases were $p16^{cons+}$ all with >50% expression. Most (96.7%) of $p16^{cons-}$ cases had <25% expression. There was complete agreement between 3 observers in assessing $p16^{\%}$ in 76% of cases and disagreement in only 1%. An even higher agreement was seen in overall p16 scoring (k 0.96-0.98) regardless of observer experience. Compared with the original interpretation, there was 96% concordance and 4% discordant results (5 $p16^{+}ISH^{-}$ and 3 $p16^{-}ISH^{+}$). p16 was expressed in 17% OSCC, 74% OPSCC, 30% OOPSCC and 17% other HNSCC, and in 81% NKSCC vs 20% KSCC ($p<0.001$); the latter were most frequently OPSCC. 64% OPSCC were $p16^{+}$ in smokers and 100% in nonsmokers ($p<0.006$) whereas 18% non-OPSCC were $p16^{+}$ in smokers and only 9% in nonsmokers (NS).

Conclusions: This study confirms excellent interobserver agreement in scoring p16 and would suggest 50% as an appropriate cutoff for positive reaction. Most $p16^{+}$ tumors are NKSCC but many OPSCC show keratinization and this cannot be used alone as a selection criterion for testing. Tumors adjacent to OP show higher p16 expression then other HN sites which may explain OSCC p16 expression. The relatively high rate of $p16^{+}$ in nonoropharyngeal SCC confirm the need for additional testing to identify false positive results.

1341 Primary Mucosal Melanoma of the Aerodigestive Tract, a Clinicopathologic and Immunohistochemical Study of 35 Cases

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Background: Primary mucosal melanoma of the aerodigestive tract (PMMAT) is a rare disease with poor survival and high rates of local recurrence and metastasis. Intraepithelial mucosal melanocytic proliferations associated with invasive melanoma may have distinctive diagnostic and prognostic features. The objective of this study was to evaluate the presence of mucosal malignant melanoma in situ (MMMIS) and perform a clinicopathologic review of PMMAT at our institution.

Design: The clinical and histopathologic features of PMMAT in 35 patients were retrospectively analyzed. PMMAT cases and five normal controls were evaluated immunohistochemically for microphthalmia-associated transcription factor (MITF).

Results: PMMAT locations were sinonasal (32), oral (2), and laryngeal (1). Patient age was 30 to 90 (median 69 years) and the M:F ratio was 3:2. Of the 26 patients with clinical followup (range: 5 to 137 months, mean 32 months), 7 were alive with no evidence of disease (ANED), 7 alive with recurrent and/or metastatic disease (AWD), and 12 had melanoma-associated death (DOD). Histologic examination revealed inter- and intratumoral heterogeneity, including epithelioid, spindled, and small cell morphology. Determination of thickness was not possible in the majority of cases due to fragmentation. MMMIS, defined as a confluent intraepithelial melanocytic proliferation of cytologically atypical melanocytes, was identified in 21/33 cases with available epithelium. MMMIS within respiratory epithelium or glands was often subtle and difficult to identify on H&E. In all cases of MMMIS, MITF staining confirmed the presence of a confluent cytologically atypical intraepithelial proliferation of melanocytes. Intraepithelial melanocytic proliferations without confluent growth were considered as hyperplasia (6 cases); 2 of which showed cytologic atypia, and 4 showed no cytologic atypia. Followup of cases with associated MMMIS (mean followup 29 months) revealed 3 with NED, 3 AWD, and 10 DOD, while those without associated MMMIS (mean followup 33 months) consisted of 4 with NED, 3 AWD, and 2 DOD (one case lacked epithelium). However, this survival difference was not statistically significant ($p>0.05$).

Conclusions: PMMAT is a rare disease with a poor prognosis. MMMIS, highlighted by MITF, is often associated with PMMAT. While this retrospective evaluation suggests a worse prognosis for patients with PMMAT and associated MMMIS, further studies are required to establish the clinical importance of this finding, especially its potential relevance to the evaluation of surgical margins.

1342 Epstein-Barr Virus-Infected Mucosal Epithelial Cells in Normals and Squamous Cell Carcinoma of the Base of Tongue and Tonsils

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Background: In the oropharynx, the base of tongue and tonsils (BOT/T) are areas that are susceptible to risk factors and prone to developing squamous cell carcinoma (SCC). Epstein-Barr virus (EBV) infection is very common in adults with seroprevalence rates in excess of 90% worldwide. Repeat infection and amplification of virus by replication in epithelial cells has been associated with both epithelial and B cell malignancies. Although there are many reports of EBV in oral mucosa, the study of EBV-infected epithelial cells in BOT/T is limited. We have analyzed expression of EBV-encoded RNA (EBER) and latent membrane protein (LMP) in the epithelial cells in normal mucosa and SCC in the BOT/T region.

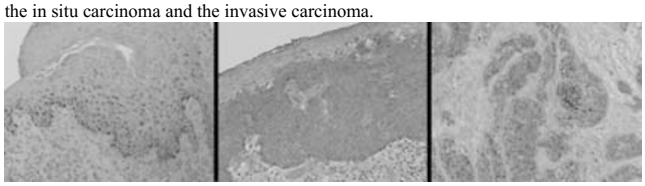
Design: Cases of BOT/T biopsies or resections, including 48 benign and 220 SCC, were selected from our archive materials from 2000 to 2012. Four-micron sections were prepared. EBER in-situ hybridization and LMP immunohistochemical stains were performed following standard laboratory protocols.

Results: Ten of the 48 benign BOT/T squamous mucosa were positive for EBER and 1 of EBER positive cases was positive for LMP. In the SCC group, 64 of 220 cases were positive for EBER and 14 of the 58 EBER positive cases were positive for LMP.

EBER and LMP expression

Cases	EBER (%)	LMP (%)
BOT/T benign squamous mucosa	10/48 (20.8 %)	1/10 (10%)
BOT/T SCC	64/220 (29.1%)	14/58 (24.1%)

In the SCC group, EBER positive epithelial cells were seen in the normal epithelium, the in situ carcinoma and the invasive carcinoma.



Conclusions: EBER positive cases were only slightly increased in the SCC group over the benign group. However, positive LMP expression was much higher in EBER positive cases in the SCC group than in the benign group. These findings suggest that EBV-infection in BOT/T epithelial cell in SCC group has a high potential for acquiring an oncogenic capacity. Other risk factors may promote the persistence of EBV in a latent phase in epithelial cells and work in concert with EBV to induce carcinogenesis.

1343 *NAB2-STAT6* Fusion and STAT6 Immunohistochemistry Help Differentiating Sinonasal Hemangiopericytoma-Like Tumors from Solitary Fibrous Tumor

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Background: Sinonasal HPC-like tumor (glomangiopericytoma) is a distinct benign neoplasm with perivascular myoid differentiation. Its genetic signature has not been yet defined and it remains debatable if it shares any pathogenetic relationship with other morphologically similar tumors, such as solitary fibrous tumor (SFT), glomus tumor and pericytoma. As recent genetic abnormalities have been described in these related tumors, such as *NAB2-STAT6* fusions in SFT, *miR143-NOTCH* fusions in glomus tumors and *GLI1* rearrangements in pericytoma, we sought to investigate a group of sino-nasal lesions with hemangiopericytic phenotype to establish a more objective classification based on molecular markers.

Design: RT-PCR for *NAB2-STAT6* with target sequencing for validation, and STAT6 IHC was performed on 5 SMA-positive sinonasal HPC-like tumors and 3 benign SFTs of sinonasal area (supported by CD34 staining). These tests were also performed in a control group, comprised of 3 benign SFTs, 5 malignant SFTs (including 2 dedifferentiated SFTs), 1 mammary myofibroblastoma, 2 infantile myofibromatoses, and 2 angioleiomyomas. In addition, FISH for *NOTCH2, NOTCH3* and *GLI1* was performed on the 5 HPC-like tumors.

Results: *NAB2-STAT6* gene fusion and STAT6 immunoreactivity was detected only in the CD34-positive SFT lesions, benign or malignant, regardless of anatomic location. Conventional and dedifferentiated areas of dedifferentiated SFTs that were interrogated separately were also positive for *NAB2-STAT6* fusion and STAT6 overexpression. Sinonasal HPC-like tumors were negative for *NAB2-STAT6* gene fusion or STAT6 by IHC, as well as additional non-SFT controls. Furthermore, no gene abnormalities were identified in *NOTCH* or *GLI1* in the sino-nasal HPC-like tumors tested.

Conclusions: The presence of *NAB2-STAT6* gene fusion plays a key role in unifying the concept of SFT at all anatomic locations. Our findings confirm that sinonasal HPC-like tumor is a distinct entity from all other morphologic mimics, of which molecular characterization is yet to be determined. The presence of an SFT-like lesion arising in the sinonasal location can be confirmed in difficult cases by either *NAB2-STAT6* RT-PCR or STAT6 reactivity.

1344 RNA Massively Parallel Sequencing Analysis of Acinic Cell Carcinomas and Polymorphous Low-Grade Adenocarcinomas of the Salivary Glands

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Background: Recurrent translocations have been shown to be a feature of numerous malignancies. A subset of salivary gland tumors has recently been found to harbor highly recurrent translocations (e.g. mucoepidermoid, adenoid cystic, mammary analog secretory, and clear cell carcinomas). Using massively parallel RNA sequencing, we sought to define whether acinic cell carcinomas and polymorphous low-grade adenocarcinomas of the salivary glands would be underpinned by recurrent fusion genes.

Design: Six fresh/frozen acinic cell carcinomas and 5 polymorphous low-grade adenocarcinomas of the salivary glands were retrieved from the Tumor Procurement Service of Memorial Sloan-Kettering Cancer Center. All cases were reviewed by three pathologists. RNA was extracted from microdissected representative frozen sections and subjected to paired-end RNA sequencing (Illumina HiSeq2000). Expressed fusion genes and 'readthroughs' were identified using a combination of Chimerascan and deFuse algorithms. To filter common RNA sequencing artifacts, fusion transcripts and 'readthroughs' identified in 47 normal samples were removed from the results of the analyses of salivary gland tumors.

Results: In acinic cell carcinomas, Chimerascan identified 388 and 14 expressed fusion genes and 'readthroughs', respectively, whereas deFuse identified 128 and 46 expressed fusion genes and 'readthroughs', respectively. Only six expressed fusion genes were identified by both approaches (*MYL12B-NCAPG*, *OXR1-NR4A3*, *MAML2-ST3GAL4*, *PIK3R3-EPB41*, *NEBL-MACROD2* and *LPHN2-ABCA4*) and none was found in >1 case. No case harbored the *ETV6-NTRK3* fusion gene characteristic of mammary analogue secretory carcinomas. In polymorphous low-grade adenocarcinomas, Chimerascan identified 92 and 36 expressed fusion genes and 'readthroughs' respectively, whereas deFuse identified 22 and 65 expressed fusion genes and 'readthroughs' respectively. Only three expressed fusion genes (*ACTN4-PRKD2*, *TFG-GPR128* and *NCALD-PLAG1*) and two 'readthroughs' (*PPP1R1B-STARD3* and *PRPSAP1-QRICH2*) were identified by both approaches. The *PPP1R1B-STARD3* was found in two out of the five cases sequenced.

Conclusions: Unlike other types of salivary gland tumors, acinic cell carcinomas and polymorphous low-grade adenocarcinomas of the salivary gland are not driven by highly recurrent fusion genes. We also confirmed the previous observations that acinic cell carcinomas of the salivary glands lack the *ETV6-NTRK3* fusion gene.

1345 Tales from the Crypt: In Search of Tonsillar Squamous Cell Carcinoma Precursor Lesions

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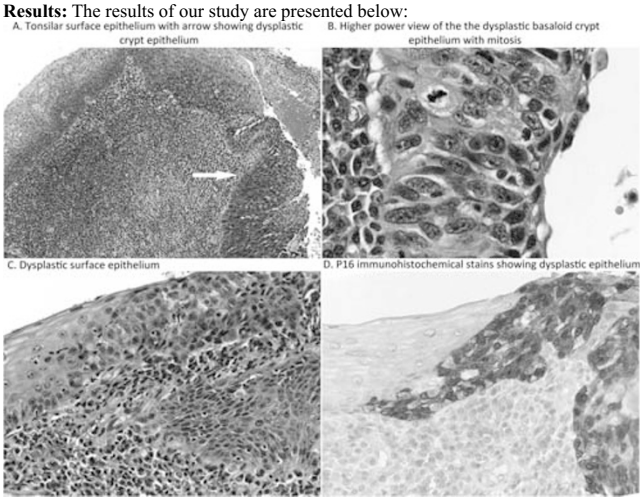
Background: The majority of squamous cell carcinomas of the tonsil are HPV-related; however, in contrast with HPV-related cervical squamous cell carcinomas, no distinct precursor lesions have been identified. It has been hypothesized that the precursor lesions occur deep in the tonsillar crypts and are therefore difficult to appreciate. Nonetheless, to our knowledge no evidence of the existence of such lesions has been reported.

Design: 51 consecutive tonsillar squamous cell carcinoma cases diagnosed between 2010 to present were identified. All cases were stained for p16, which was used as a surrogate marker for HPV. The presence of continuous block-like nuclear and cytoplasmic staining was considered positive; weak, patchy staining was considered negative. Weak diffuse staining with focal accentuations was considered equivocal. Residual surface or crypt epithelium was identified and surface epithelial dysplasia and dysplasia involving the crypts was assessed separately. If identified, squamous dysplasia was classified as basaloid and differentiated and graded according to the upper aerodigestive tract grading scheme. Squamous cell carcinomas were classified into keratinizing and nonkeratinizing.

Results: The results of our study are presented below:



Tonsillar squamous cell carcinoma cases demographics and morphologic features

	Age (mean)	Gender	p16	Dysplasia on surface and (Percent)	Residual Crypts	Crypt Dysplasia and (Percent)
non-keratinizing (n=38)	58.5	M=34, F=4	POS:34	13, (38%)	14	6, (43%)
			NEG:4	2, (50%)	2	0, (0%)
keratinizing (n=13)	60	M=8, F=5	POS:7	3, (43%)	3	0, (0%)
			NEG:6	5, (83%)	1	0, (0%)
benign (n=9)	45	M=5, F=4	POS:0	0, (0%)	0	0, (0%)
			NEG:9	0, (0%)	9	0, (0%)

Conclusions: Dysplasia involving tonsillar crypts is only rarely identified in patients with squamous cell carcinomas, most likely because the invasive squamous cell carcinomas overgrow the precursor lesions but may preserve the outline of the crypts. Crypt dysplasia is present in p16 positive non-keratinizing squamous cell carcinomas. Surface dysplasia is more frequently seen in keratinizing p16 negative squamous cell carcinomas. A larger number of benign tonsils need to be studied to assess the frequency of dysplasia in non-tumor bearing tonsils.

1346 A Systematic Review of Oral and Maxillofacial Pathology found in Children: 2000-2010

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Background: To systematically review and characterize all the oral and maxillofacial lesions biopsied in pediatric patients at Columbia University Medical Center.

Design: Biopsied cases from a pediatric population (0-18 year old) from 2000-2010 were retrieved from the Oral Pathology Department of our institution. Oral and Maxillofacial diagnoses were grouped into nine categories, and lesions were evaluated for age, gender, anatomic location and pathological diagnosis. Data was analyzed using descriptive statistics.

Results: A total of 3150 oral and maxillofacial biopsies were recorded for patients between 0- 18 years from a total of 48,268 adult and pediatric biopsies over the ten year time period. The diagnostic category with the largest number of specimens was odontogenic cysts and periapical pathology. The most common salivary lesion was mucocele. There were three cases of squamous cell carcinoma and one case of mucoepidermoid carcinoma in this pediatric population. No other malignant lesions were noted. Additionally one case of sarcoidosis was noted.

Conclusions: Although the vast majority of pathology encountered in this cohort was benign, many lesions required intervention to improve quality of life for the patient. Therefore early recognition of oral lesions in children is critical and a complete oral examination should be an integral part of a child's well visits. Furthermore the degree and the nature of the lesions found in this cohort corroborate American Academy of Pediatrics' recent efforts to include oral health screenings in child well visits.

1347 Characterization of Two Distinct Precursor Lesions of Squamous Cell Carcinoma of the Head and Neck Skin in Kidney Transplant Patients

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Background: Renal transplant recipients are highly susceptible to skin cancer in sun exposed areas of the body particularly the head and neck skin. Immune suppression, ultraviolet light exposure and cutaneous HPV (HPV β) infection are believed to be important etiologic factors. In an attempt to further clarify the pathogenic mechanisms involved in the process of malignant transformation we investigated the morphologic features and immunophenotypic characteristics of premalignant skin lesions in a cohort of kidney transplant patients.

Design: Sections of skin biopsies taken from 37 kidney transplant recipients were examined microscopically. Fifty nine premalignant lesions were identified and were immunohistochemically stained for p16, p53, Ki-67 and Bcl-2. p16 was considered negative if areas of nuclear and cytoplasmic staining were < 25%, while 25-50%, 50-

75% and >75% were scored as 1+,2+ and 3+ respectively. Positive staining for p53 and Ki-67 was scored as 1+, <25%, 2+, 25-50% and 3+, > 50%. Bcl-2 was scored as positive, > 10% or negative, < 10 %.

Results: The average numbers of invasive squamous cell carcinoma and premalignant skin lesions that developed during≥ 5 years post transplantation period were 6.4 and 5.1 per patient respectively. Two morphologically distinct types of precursor lesions were identified; dysplasia with Bowenoid features (BD) (31 cases) and premalignant keratosis without Bowenoid features (NBD) including hypertrophic and atrophic actinic keratosis (28 cases). The immunohistochemical profiles differed considerably between the two types of lesions with regard to p16, Ki-67 and Bcl-2 [Table 1] while no difference was observed in p53 staining.

Table 1

IHC stain	p16		Ki-67		Bcl-2	
	≥2+ no. (%)	- and 1+ no.(%)	≥2+ no(%)	1+ no (%)	Positive	Negative
BD	25 (86)	4 (14)	27 (93)	2 (7)	25 (86)	4 (14)
NBD	3 (12)	23 (88)	7 (27)	19 (73)	7 (27)	19 (73)
p value	<0.0001		<0.0001		<0.0001	

Conclusions: The observed differences in morphologic features and immunohistochemical profiles between BD and NBD are highly suggestive of distinct etiology and pathogenesis. p16 over expression and higher Ki-67 labeling scores in BD are consistent with a relationship to HPV analogous to that observed in mucosal lesions . Strong and diffuse p16 staining could possibly be used as a surrogate marker for HPV-related skin lesions similar to the mucosal ones. However, unlike HPV-related mucosal lesions of the head and neck, there was no significant difference in p53 expression. Bcl-2 over expression may be a unique feature of the cutaneous lesions.

1348 Extent of Invasion and Prognosis in Salivary Carcinoma Ex-Pleomorphic Adenoma

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Background: Carcinoma ex-pleomorphic adenoma (CPA) is defined as a pleomorphic adenoma (PA) from which a malignancy is derived. It is stratified according to the extension of penetration of the malignant component into non invasive, minimally invasive and invasive. The first two groups have an excellent prognosis, similar to that of a PA, while the latter has a more guarded prognosis. The WHO classification of Salivary Gland tumors defines 1.5mm invasion as the threshold to distinguish frankly invasive carcinomas. Nevertheless, this is a widely disputed matter as others suggest that a higher threshold (4-6mm) should be accepted. To contribute to this discussion we studied a series of 58 cases of CPA aiming at evaluating which extra-capsular extension threshold impacted on prognosis.

Design: Fifty-eight cases of CPA, diagnosed between 1982 and 2013, were reviewed. Clinical data was obtained from patient's charts. Measurement of the extent of invasion used a calibrated low-power objective for tumors under 3mm and a transparent ruler placed directly onto the slide for the remaining, perpendicularly to the capsule of the pre-existing PA, and was expressed in mm. Eight cases were excluded due to fragmentation of the specimen.

Results: No metastases or deaths occurred in non invasive cases or with invasion ≤1.5mm. Twenty-nine cases had between 2.5mm and 12mm extent of invasion, 11 with disease progression and of these 9 died. This group had a significantly different disease-free and overall survival. There were no cases with invasion >1.5mm and <2.5mm.

Conclusions: Our data shows that patients with ≤1.5mm extent of invasion have good prognosis. The minimum extent of invasion that resulted in death was 2.5mm, not in agreement to the findings of recently published series. Of note, 18 cases with≥2.5mm did well, confirming that others factors beside the extent should be considered in the clinical management of these patients. Due to the fact that we did not have cases with >1.5mm and <2.5mm extension we cannot suggest a higher threshold than the one considered by the WHO.

1349 Polymorphous Low Grade Adenocarcinoma Has a Consistent p63+/p40– Immunophenotype That Helps Distinguish It from Adenoid Cystic Carcinoma and Cellular Pleomorphic Adenoma

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Background: Polymorphous low grade adenocarcinoma (PLGA) is a tumor of minor salivary glands that exhibits considerable morphologic overlap with adenoid cystic carcinoma (ACC) and cellular pleomorphic adenoma (PA), especially in small biopsy specimens. Unlike these other tumor types, PLGAs do not harbor a myoepithelial component; yet their frequent positivity for p63 diminishes the usefulness of this particular myoepithelial marker as a discriminating immunostain. P40 is an antibody that recognizes ΔNp63, a p63 isoform that is more specific than p63 for true myoepithelial differentiation. As such, p40 immunostaining could help distinguish PLGAs from ACCs and PAs.

Design: P63 (4A4; BioCare Medical, Concord CA) and p40 (BC28, Biocare) immunohistochemistry was performed on paraffin embedded, formalin fixed tissue from 11 PLGAs, 101 ACCs, and 31 PAs. Eight of the PLGAs were stained on whole sections, while the remaining tumors were present on tissue microarrays. Antigen retrieval with high pH buffer was followed by primary antibody incubation for 32 minutes. Staining was performed on an automated stainer using an Ultra View detection as per manufacturer's instructions (BenchMark Ultra, Ventana Medical Systems Inc., Tucson AZ).

Results: All 11 PLGAs (100%) were positive for p63 but completely negative for p40. Among ACCs, 91 of 101 (90%) were positive for p63 and 90 of 101 (89%) were positive for p40. The single discordant p63+/p40– ACC exhibited solid architecture and high grade features not typically seen in PLGA. Among PAs, 21 of 31 (68%) were positive for p63 and 13 of 31 (42%) were positive for p40. For the PAs, the discordant p63+/p40– staining pattern was seen only in the chondromyxoid stroma. The cellular ductal component of the PAs were consistently either p63+/p40+ or p63–/p40–.

Conclusions: PLGA consistently exhibits a p63+/p40– immunophenotype that can help distinguish it from ACC and cellular PA, tumors that characteristically demonstrate concordant p63 and p40 immunostaining patterns. A p63/p40 immunohistochemical panel can provide a valuable tool for making the distinction between these morphologically similar but clinically divergent entities.

1350 Alcohol-Dysregulated MicroRNAs in the Pathogenesis of Oropharyngeal Cancer

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Background: Though alcohol consumption has been implicated in the pathogenesis of oropharyngeal cancer, the molecular mechanism for the pathogenesis and progression of alcohol-related oropharyngeal cancer remains poorly understood. Recent advances in the discovery and understanding of non-coding RNAs (ncRNAs) have indicated that these RNAs are not transcriptional noise, but rather have critical roles as transcriptional and post-transcriptional regulators and as guides for chromatin-modifying complexes. MicroRNAs are one of the key ncRNAs that have a profound effect on a myriad of biological processes including oncogenesis and tumorigenesis. Our central hypothesis is that alcohol-mediated dysregulation of specific microRNAs is critical to the pathogenesis and progression of oropharyngeal carcinoma.

Design: Using next generation sequencing analysis, we analyzed the non-coding RNA of 136 head and neck squamous cell carcinoma (HNSCC) patients. In order to further investigate these microRNAs, we exposed a panel of normal oral epithelial cell cultures/cell lines to low, medium, and high levels of ethanol (.1 to 1% by volume) for 4 weeks to mimic social, moderate, and heavy drinkers, respectively and performed qPCR microRNA array to determine alcohol regulation of microRNAs *in vitro*. We then performed functional assays on miR-30a-5p and miR-934.

Results: We have identified a panel of 8 microRNAs that are differentially expressed between alcohol drinkers and non-drinkers from analysis of RNAseq clinical data. In our *in vitro* experiments, we have identified microRNAs that are differentially regulated as a result of alcohol exposure, including those identified from the clinical data. Of the microRNAs identified, we have selected miR-30a-5p and miR-934 for further investigation. Our preliminary data indicate that ectopic expression of both these microRNAs resulted in increased expression of the stem cell genes, CD44, Oct3/4, Nanog, BMI-1. Furthermore, forced expression of these microRNAs resulted in increased proliferation and inhibition of cisplatin-induced apoptosis signifying the malignant transformation of normal oral cells.

Conclusions: Our results indicate that the identification of key differences in microRNA expression between control and alcohol-exposed cells will result in discovering genes and events associated with oral cancer initiation and progression. Potential key microRNAs could serve both as prognostic indicators and therapeutic targets, leading to more effective treatments for alcohol-associated oropharyngeal cancer.

1351 DOG1, p63, and S100 Protein: A Novel Immunohistochemical (IHC) Panel in the Differential Diagnosis of Oncocytic Salivary Gland Neoplasms

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Background: Oncocytic features are commonly identified in salivary gland neoplasms, which include Warthin's tumor (WT), acinic cell carcinoma (AciCC), mucoepidermoid carcinoma (MEC) and oncocytoma (ONC). This study utilized a DOG-1, P63 and S-100 IHC staining panel to subclassify these salivary gland neoplasms with oncocytic differentiation.

Design: 31 Fine Needle Aspiration Cell Blocks (CB) of oncocytic salivary gland neoplasms (16 WT, 10 AciCC, 3 MEC, and 2 oncocytoma [ONC], and 75 salivary gland resections (7 WT, 27 AciCC, 36 MEC, 2 high grade adenocarcinomas [ADC], 2 ONC, and 1 papillary cystadenoma [PC]), were immunostained for DOG-1, p63, and S100.

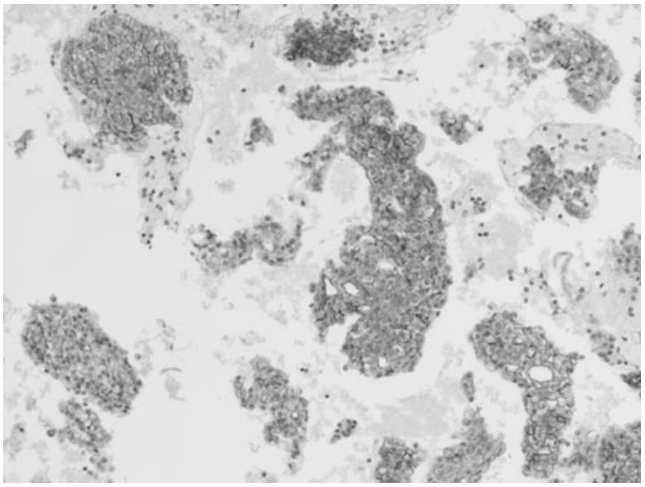
Results:

Cytology Data

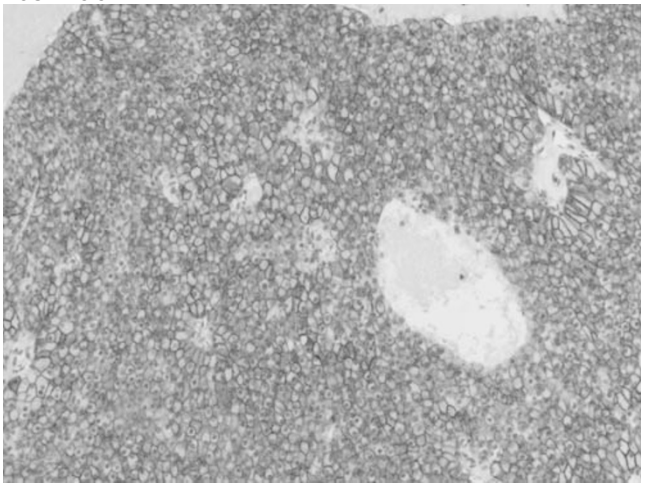
N= 31	DOG-1 + (%)	P63 + (%)	S-100 + (%)
WT (16)	0/16 (0%)	14/16 (87.5%)	0/16 (0%)
AciCC (10)	7/10 (70%)	0/10 (0%)	0/10 (0%)
MEC (3)	0/3 (0%)	3/3 (100%)	0/3 (0%)
ONC (2)	0/2 (0%)	1/2 (50%)	0/2 (0%)

Surgical Resection Data

N= 75	DOG-1 + (%)	P63 + (%)	S-100 + (%)
WT (7)	0/7 (0%)	6/7 (86%)	0/7 (0%)
AciCC (27)	25/27 (93%)	3/27 (11%)	2/27 (7%)
MEC (36)	19/36 (53%)	35/36 (97%)	0/36 (0%)
ADC (2)	0/2 (0%)	1/2 (50%)	0/2 (0%)
ONC (2)	0/2 (0%)	2/2 (100%)	0/2 (0%)
PC (1)	1/1 (100%)	1/1 (100%)	0/1 (0%)



DOG1 IHC CB



DOG1 IHC Resection

Conclusions: DOG1 and p63 were very useful in distinguishing AciCC from WT on CBs, since 100% of WT were DOG1 – and 87.5% were p63 +, whereas 70% of AciCC were DOG1 + and 100% were p63 -. The resection results correlated with those on CB's: 100% WTs were DOG1 – and 86% were p63 +, whereas 93% of AciCCs were DOG1 + and 89% were p63 -. S-100 and DOG1 are negative in both WT and ONC, with <10% S100 positivity in AciCC. The pitfalls to be aware of when using DOG1 to subclassify an oncocytic neoplasm include DOG1 + staining in normal salivary gland acini and focal positive staining in MEC. In summary, an IHC panel including DOG1, p63, and S100 can significantly decrease false negative cases in oncocytic salivary gland neoplasms.

1352 Extrapleural Solitary Fibrous Tumor of Head and Neck

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Background: Extrapleural solitary fibrous tumors (ESFT) are a well recognized entity. These mesenchymal tumors of fibroblastic type are characterized by the prominent hemangiopericytoma-like branching vascular pattern and a spindle cell proliferation. These lesions are uncommon in the head and neck. ESFT are generally benign slow-growing neoplasms that can be successfully treated by complete excision. Malignant ESFT is reported in about 10%. We report 14 cases of ESFT arising in the head and neck encountered in our institution.

Design: Fourteen patients with ESFT were identified from the pathology files at our institution in a 12-years period (from 1999 to 2011) under an IRB approved protocol. The clinical presentation, histopathological findings, and literature on Head and Neck ESFT were reviewed.

Results: The patients were 6 males and 8 females, ranging from 24 to 81 years-old (mean age: 52.64). Histologically, the tumors showed a predominant patternless architecture, spindle cell pattern, fibro-collagenous stroma, and hemangiopericytoma-like vascular pattern. Most of the tumors showed CD34 expression (64%, 9/13). Other immunoreactivities included vimentin (7/7), bcl-2 (4/4), CD99 (3/6), smooth muscle actin (4/10), and S-100 (1/12). The four malignant ESFT, 28.5% of all cases, showed cytological atypia (4/4), necrosis (2/4) and higher mitotic counts (4 or more mitosis per 10 high power fields). Three of 4 malignant ESFT had local recurrence; one of them showed distant metastasis.

Conclusions: In our series of ESFT arising in the head and neck, malignant ESFT is found more often than previously reported.

1353 *EWSR1* Genetic Rearrangement Is Present in High Grade Clear Cell Myoepithelial Carcinoma of Salivary Glands

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Background: The Ewing sarcoma breakpoint region 1 *(EWSR1)* is translocated in many sarcomas. Recently, its rearrangement has been described in salivary hyalinizing clear cell carcinoma and a subset of soft tissue myoepitelial tumors, but not in their salivary gland myoepithelial counterparts.

Design: This study examines the presence of the *EWSR1* rearrangement in a variety of salivary gland tumors with myoepithelial differentiation. A total of 22 cases included 4 cases of high grade (HG) clear cell myoepithelial carcinomas (MECa), 5 cases of low grade (LG) MECa, 8 cases of epithelial-myoepithelial (EMECa) or hybrid EMECa/MECa, and 5 cases of MECa ex pleomorphic adenoma. All the tumors in this spectrum were reviewed, reclassified and tested by FISH for the *EWSR1* rearrangement using the Vysis *EWSR1* Break Apart FISH Probe Kit. Cut off value was set to 10% of nuclei with chromosomal breakpoint signals.

Results: The *EWSR1* rearrangement was detected in all three analazyble cases of four HG clear cell MECa, the remaing one case had low quality DNA. All other myoepithelial salivary gland tumors had intact *EWSR1*. Three patients with HG clear cell MECa with rearranged *EWSR1* included two women and one man, ages ranged from 43 to 60, and locations included parotid gland (2) and palate (1). All three *EWSR1* rearranged tumor had identical histomorphology. They were arranged in nodules composed of compact nests of large polyhedral cells with abundant clear cytoplasm. Large comedo necroses and high proliferative activity with MIB1 index 40 to 90% were seen in all cases. All tested tumors were immunoreactive with antibodies to p63, smooth muscle actin, S-100 protein, calponin, CK 14, and SOX10. All three were large tumors with skin invasion (2); in two patients distant metastases to lung and soft tissue of head and neck developed, and one patient died of the tumor within 12 months follow-up period.

Conclusions: We describe for the first time *EWSR1* gene rearrangement in a subset of myoepithelial carcinomas of salivary gland. The *EWSR1* rearranged MECa represent distinctive aggressive variant composed predominantly of clear cells with large comedo necroses in the central parts of tumor nests, characterized by poor clinical outcome.

1354 Racial Disparity in p16 Positive oropharyngeal Squamous Cell Carcinoma

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Background: Studies have definitively established a causal relationship between HPV and squamous cell carcinoma of the oropharynx. Up to 95% of HPV positive OPSCC patients are also p16 positive by immunohistochemical (IHC) methods. Previous research has indicated that HPV16 positive OPSCC patients have a more favorable prognosis. For this reason, p16 status is emerging as an important consideration during risk stratification when predicting OPSCC patient survival. The majority of studies indicating that p16+ OPSCC patient prognosis is improved are based on research designed to represent a general population. This study examines the influence of p16 positivity on patient prognosis in different racial/ethnic groups.

Design: A retrospective cohort of OPSCC was designed based on history and outcomes for each of the patients. Overall survival (OS) was measured based on months of survival from time of diagnosis to death. Censored data was used to consider patients followed for different lengths of time or lost to follow up. 120 patients with known p16 status of which 60 African-American (AA) and 60 Caucasian (CA) were studied. The 2 groups were matched for stage of disease. Kaplan Meier survival curves were produced to compare OS between 60 AA (31 p16+, 29 p16 -) and 60 CA (35 p16+, 25p16-). The Cox Proportional Model was used in a multivariate analysis in comparing these groups while controlling for gender, age, tobacco use history, stage of disease at diagnosis and the type of treatment received. SPSS version 21 was used for final statistical analysis.

Results: CA patients, on univariate analysis showed improved overall survival in p16+ individuals ($p < .0001$). A multivariate analysis also indicated statistically significant differences between p16+ and p16- CA and showed improved survival for those that were p16 + ($p = .004$). AA patients did not show improved survival when p16 positive, by univariate analysis ($p = .612$) or multivariate analysis ($p = .645$).

Conclusions: 1. This study is in concordance with existing data in the literature, showing improved survival for p16 positive patients (only in this studied CA group).
2. However, the benefit of improved survival for p16+ OPSCC was unexpectedly lost in AA patients although receiving the same treatment.
3. When treating patients with OPSCC racial/ethnic factors should be considered, in addition to p16 status.

1355 TGF-beta and Its Association with Mast Cells in Actinic Cheilitis

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Background: Transforming growth factor beta (TGF-beta) plays a key role in cancer progression by stimulating an immunosuppressive microenvironment. Mast cells produce TGF-beta and also stimulate its expression by other cells. Since mast cells are increased in actinic cheilitis (AC), a premalignant lesion of the lip, the aim of this study was to characterize the expression of TGF-beta in AC and to assess if there is

an association between mast cell density and the expression TGF-beta at the earlier stages of lip carcinogenesis.

Design: NL (n=18) and AC (n=69) samples were obtained from the archives of the Oral Biology and Pathology laboratory of the University of Concepcion, College of Dentistry. Samples were processed for immunohistochemical detection of TGF-beta and tryptase-positive mast cells. A score of epithelial expression of TGF-beta (intensity by extension) and dichotomic TGF-beta expression (TGF-high and TGF-low) using the median score as the cut off value were obtained. Mast cells were quantified at intraepithelial, papillary and reticular stroma (30 fields, 400x). Expression of TGF-beta1 mRNA was measured by qRT-PCR in 14 AC samples and compared to normal oral mucosa, using GAPDH as the housekeeping gene. Double immunofluorescence was used to assess co-localized expression of TGF-beta with mast cells by confocal microscopy (CMA Bio Bio).

Results: Epithelial TGF-beta expression was significantly increased in AC as compared to NL ($P < 0.001$, Mann-Whitney). In addition, a 4-fold increase in TGF-beta1 mRNA was found in AC as compared to normal oral mucosa. Mast cell density was increased in papillary and reticular stroma of AC as compared to NL ($P < 0.001$), with no differences in intraepithelial mast cell infiltration between NL and AC. AC and NL samples with a high expression of TGF-beta had significantly higher mast cell density at the papillary stroma than samples with low TGF-beta expression ($P<0.05$, Fisher´s Exact Test). Mast cells were positive for TGF-beta in AC as determined by confocal microscopy.

Conclusions: The results suggest that there is an association between the increased expression of TGF-beta and the density of mast cells at the earlier stages of lip carcinogenesis, suggesting an immunomodulatory role of mast cells that could favor cancer progression. Supported by Grant FONDECYT 1090287, CONICYT Chile.

1356 Mammary Analogue Secretory Carcinoma (MASC) and Look-Alikes: Don't Count on Mamma!

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Background: MASC is a recently recognized low-grade salivary carcinoma characterized by an ETV6 rearrangement. Before its recognition, MASC was frequently classified as acinic cell carcinoma (AcCC). Mammaglobin (Mamma) & S100-protein are useful in distinguishing MASC from AcCC. Low-Grade Salivary Duct Carcinoma (LGSDC) also histologically overlaps with MASC. However, LGSDC & High-Grade Salivary Duct Carcinoma (HGSDC), a LGSDC-look-alike, have not been characterized with respect to Mamma. Here we examine IHC and gene profiles in the context of distinguishing MASC from look-alikes.

Design: 37 cases were studied (Table 1) for Mamma, S100, DOG1, & GATA-3 expression by IHC, HER2 (CISH) & ETV6 (FISH).

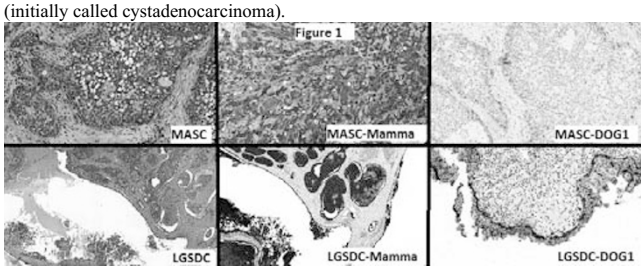
Results:

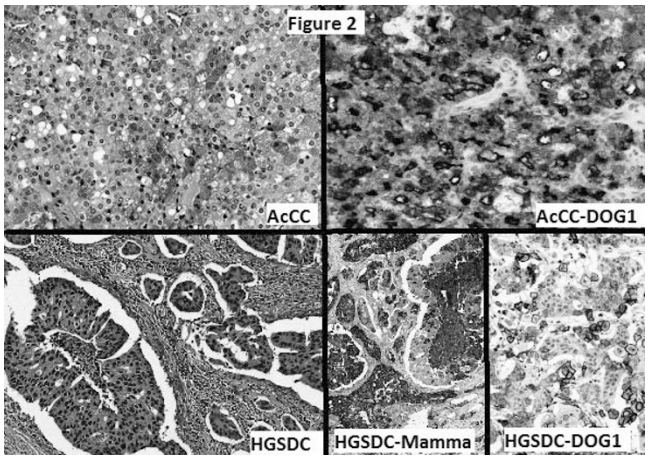
Table 1

	S100	Mamma	DOG1	GATA3	HER2 CISH	ETV6 FISH
MASC (12) (Fig1)	11/12	12/12	5/5 m	5/5 n	0/7	4/4
LGSDC (5) (Fig1)	5/5	5/5	5/5 p	1/1 n	0/4	pd
AcCC (8) (Fig2)	0/8	0/8	4/4 ap, c	2/6 n	pd	pd
HGSDC (12) (Fig2)	0/11	8/12	4/5 m	9/9 n	8/10	pd

m = membranous, p = peripheral, ap = apical, c=cytoplasmic, n=nuclear, pd = pending

After study, 3 tumors (two called AcCC, one called undifferentiated carcinoma) were reclassified as MASC; two were Mamma+/S100+, the third was Mamma+/S100-. A recurrent Mamma+/S100-/HER2 amplified tumor was reclassified as in-situ HGSDC (initially called cystadenocarcinoma).





Conclusions: Mamma expression is not specific for MASC, as it is commonly expressed by MASC mimickers. MASC & LGSDC are best distinguished by histology. MASC is typically solid, predominantly extraductal, & composed of cells with eosinophilic bubbly cytoplasm. LGSDC is primarily intra-ductal and forms floppy, fenestrated proliferations composed of low-grade tumor cells. FISH for ETV6 rearrangement remains the gold standard in difficult cases. HER2 CISH may help separate HGSDC from mimics. Lack of GATA3 expression favors AcCC.

1357 EpCAM Is a Useful Marker in the Diagnosis of Keratocystic Odontogenic Tumor

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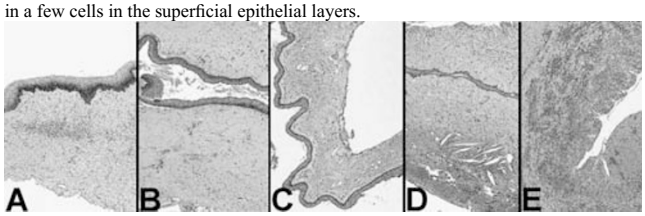
Background: Keratocystic odontogenic tumor (KCOT) is a benign uni- or multicystic, intraosseous tumor of odontogenic origin with a potential infiltrative behavior. Therefore, a correct histopathological diagnosis with the exclusion of a radicular or follicular cyst is mandatory to justify the more aggressive surgical treatment. Despite characteristic histopathological features (e.g. parakeratinized stratified squamous epithelium without rete ridges, palisading basal cells) the differential diagnosis of KCOT is sometimes difficult and raises the need of ancillary testing. Due to the fact that KCOT is in some cases part of the inherited naevoid basal cell carcinoma syndrome (NBCCS) with a strong expression of EpCAM in basal cell carcinoma, the expression of EpCAM in KCOTs, radicular and follicular cysts was examined.

Design: Diagnosed between 1993 and 2010, 128 KCOTs, 147 follicular cysts and 115 radicular cysts were retrieved from the archives of our institute and installed in 35 paraffin tissue microarrays (PTMAs) (core diameter: 4 mm). The PTMAs were stained immunohistochemically with a monoclonal mouse antibody (anti-EpCAM; clone Ber-EP4, dilution: 1:100; Dako) using an automated stainer (Benchmark, Ventana).

Results: 89% (114 cases) of the KCOTs expressed EpCAM in the squamous epithelium in a heterogeneous fashion: 35% (46 cases) displayed a weak (Figure 1 C), 42% (54 cases) a moderate (Figure 1 B) and 9,4% (12 cases) a strong basal and parabasal staining (Figure 1A). In squamous epithelium with underlying inflammation, EpCAM expression was reduced or lost. 1,6% (2 cases) showed only a superficial staining pattern. 7% (14 cases) of the KCOTs were negative for EpCAM in contrast to 70% (103 cases) of the follicular cysts (Figure 1 D) and 99% (114 cases) of the radicular cysts (Figure 1 E). Two follicular cysts (2%) displayed a weak to moderate expression in the basal/parabasal layer whereas 28% (42 cases) showed a weak to strong EpCAM expression in the superficial epithelial layers. Only one case of the radicular cysts expressed EpCAM in a few cells in the superficial epithelial layers.



Conclusions: The immunohistologically detected expression of EpCAM in the basal and parabasal layers of the squamous epithelium may be helpful in the diagnosis of KCOTs.

1358 NUT Midline Carcinoma: Morphoproteomic Characterization with Genomic and Therapeutic Correlates

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Background: NUT carcinoma is a rare entity arising primarily in the midline of teenagers and young adults. Genomically, it is associated with a mutation in the nuclear protein in testis (*NUT*) gene and most commonly, a translocation involving the *BRD4* and *NUT* genes. The resultant is a partial or near total block in differentiation of tumor cells into mature squamous elements. Such tumors are resistant to conventional therapy with a reported mean survival at less than 1 year.

Design: A series of two cases with genomic confirmation as NUT midline carcinoma were studied by morphoproteomic analysis using immunohistochemical probes for the following protein analytes: CD133 and Sox2 as markers of stemness; phosphorylated

(p)-insulin-like growth factor (IGF)-1R (Tyr1165/1166), p-mTOR (Ser 2448) and p-Akt (Ser 473) as mTORC2 pathway and promoters of stemness; and Sirt (silent mating type information regulation 2 homolog) 1 and c-Myc as molecular impediments to differentiation.

Results: Variable expression of stemness markers, CD133 and Sox2 was evident in the undifferentiated cells of the tumor and virtually absent from the squamous elements, when present. The mTORC2 pathway was constitutively activated and overexpressed in the undifferentiated cells as evident by nuclear p-mTOR (Ser 2448) and p-Akt (Ser 473) co-expression and the upstream effecter, namely p-IGF-1R (Tyr1165/1166). Sirt1 and c-Myc were highly expressed in the nuclei of the undifferentiated cell component.

Conclusions: These morphoproteomic findings coincide with the genomic and fusion protein data regarding the ability of BRD-NUT fusion protein to inhibit differentiation and promote stemness. Additionally, the presence of a constitutively activated IGF-1R/mTORC2/Akt pathway and overexpression of Sirt1 and c-Myc in NUT cases likely contribute to the stemness and the block in differentiation, respectively. The ability of histone deacetylase inhibitors and specifically, vorinostat to dephosphorylate Akt and to downregulate Sirt1 signaling; and the report by Schwartz, et al. of preclinical studies showing that vorinostat promotes differentiation and growth inhibition in NUT xenograft models and an objective clinical response in a patient with NUT midline carcinoma provides a therapeutic correlate to the morphoproteomic findings in this report.

1359 Human Papillomavirus in Oropharyngeal Squamous Cell Carcinomas in Vermont: 1989-2010

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Background: Oropharyngeal squamous cell carcinoma (OSCC) is on the rise, despite a worldwide decrease in overall incidence of head and neck squamous cell carcinoma, particularly in a younger population comparatively. Human papilloma virus (HPV) infection has been well-established as an etiologic agent of those arising from the oropharynx. As such, the rising incidence has been attributed to an increase in oral HPV infection. This study focuses on OSCC diagnosed at our institution from 1989-2010.

Design: Retrieved archival OSCC tissue blocks were assessed for presence of HPV by chromogenic *in situ* hybridization (CISH) and polymerase chain reaction (PCR) followed by sequencing of specific subtypes. Immunohistochemical staining for p16 was also performed.

Results: OSCCs from 158 patients were available for study (128 males, 30 females; average age 58 years) (Table 1). Increase in incidence of OSCC as well as those driven by HPV infection was observed over time (Figure 1). OSCCs considered truly HPV driven were defined as diffuse nuclear and cytoplasmic p16 staining with HPV detection either by PCR or CISH. HPV was detected by PCR in 124/158 (78.5%) cases, with HPV-16 as the predominant type (85.5%), followed by types 18, 35, 66, 33 and 31 (Table 2). HPV was detected by CISH in 100/121 (82.6%) cases.

Table1: Features of OSCC patients

		1989-1994 (n=24)	1995-1999 (n=26)	2000-2004 (n=41)	2005-2010 (n=67)
Male to Female ratio		3 (18:6)	5.5 (22:4)	4.9 (34:7)	4.2 (54:13)
Mean age (years)[1]	Overall	61	58	58	56
Males		62	58	58	55
Females		60	57	59	57
HPV driven		59	57	55	54
Non-HPV driven		63	62	63	59

[1] Age at diagnosis

Figure1: OSCC and HPV-driven OSCC in Vermont over time

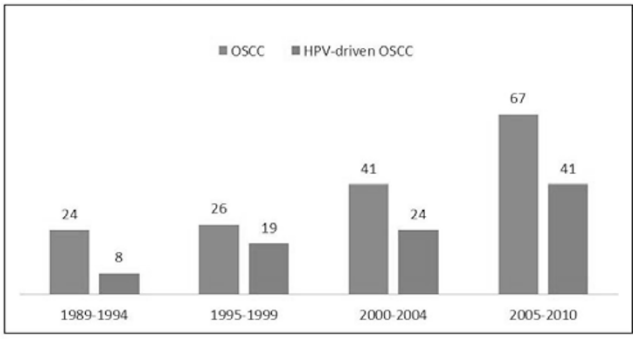

Table2: HPV types detected by PCR

	HPV + by PCR	type 16	type 18	type 35	type 66	type 33	type 31
1989-1994	18	17					1
1995-1999	24	21	2	1			
2000-2004	30	20	3	3	3	1	
2005-2010[1]	52	48		1		1	

[1] Subtype not defined in 2 cases

Conclusions: In accordance with trends seen worldwide, OSCC in the Vermont population is increasing in incidence over time and is observed in younger populations. Our data show statistical significance of HPV association with these tumors over the past two decades ($p<0.05$) suggesting HPV infection may account for the increase in OSCC. These results may lend support for preventative measures such as HPV vaccinations.

1360 Canalicular Adenoma: A Clinicopathologic and Immunohistochemical Analysis of 67 Cases

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Background: There is a lack of a comprehensive immunohistochemical (IHC) analysis of canalicular adenoma (CA) combined with unique histologic features. Given the usual small biopsies, IHC may be useful in distinguishing CA from other tumors in the differential diagnosis.

Design: Retrospective.

Results: The patients included 54 females and 13 males (4.2:1), aged 43 to 90 years, with a mean age at presentation of 70.2 years. Clinical presentation was generally a mass (n=62) slowly increasing in size (mean, 35.6 months), affecting the upper lip (n=46), buccal mucosa (n=17) or palate (n=4), involving the right (n=29), left (n=24) or midline (n=9); there were no major salivary gland tumors. The tumors ranged in size from 0.2 to 3 cm (mean=1.2 cm). Most were multinodular or bosselated (76%) surrounded by a capsule. Histologically, the tumors were characterized by cystic spaces, tumor beading, tubule formation, with luminal "balls" (n=41) and a stellate-reticulum pattern. The cells were cuboidal to columnar with stippled chromatin and inconspicuous mitoses. A myxoid stroma (n=68), luminal hemorrhage (n=55), and luminal microliths (n=33) were characteristic. CA showed the following immunohistochemistry findings:

Immunohistochemistry

Antibody	% Reactive/Type	Antibody	% Reactive/Type
CK-pan	100 (S, D, M)	CK7	100 (S, D, M)
CK20	0	CK5/6	74 (S, F, A)
CK903	88 (S, F, L)	EMA	80 (S, F, B/A)
CAM5.2	100 (S, D)	SMA	0
MSA	0	SMMHC	0
Calponin	64 (W, F)	CEAm	4 (S, D, L)
S100 protein	98 (S, D, N)	GFAP	81 (S, F, P)
CD117	91 (S/W, F)	CD10	17 (S, F, Ca)
Mammaglobin	23 (W, F)	bcl-2	43 (W, F)
p40	0	p63	74 (S, F, N and/or C)
CD34	0	CD15	98 (S, D, T)
Vimentin	83 (S, D, B)	p53	83 (W, F)
Ki-67	Range: 1-5% N		

S: Strong; W: Weak; D: Diffuse; F: Focal; L: Luminal; P: Peripheral; M: Membrane; B: Basal; A: Ball; N: Nuclear; Ca: Canalicular; C: Cytoplasmic; T: Stromal

Conclusions: CA are unique salivary gland tumors showing a distinct architecture and phenotype. They predilect to older women, with the majority multifocal and affecting the upper lip. They do not develop in the major salivary glands. The luminal squamous ball and stellate-reticulum pattern is unique, as is the cytoplasmic p63 reaction, lack of muscle markers, and peripheral GFAP reaction.

1361 Expression of PDK1, PTEN, and Stathmin in Conventional Head & Neck Squamous Cell Carcinoma: Progressive PI3K Pathway Activation in Tumors of Increasing Grade

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Background: Activation of the Phosphatidylinositol 3-Kinase (PI3K) pathway occurs in many tumor types. Expression of several pathway components, such as Akt and (to a lesser extent) PTEN, has been investigated in head & neck squamous cell carcinoma (HNSCC) by immunohistochemical staining (IHC). Expression of other components such as PDK1, as well as Stathmin, a marker of PI3K pathway activation, has not yet been evaluated in this disease. We sought to characterize expression of Stathmin, PDK1, and PTEN in conventional (non-HPV-associated) HNSCC specimens.

Design: We constructed a tissue microarray (TMA) from 30 HNSCC cases sampled or resected between 1998-2009. Tumors were graded as well-, moderately, or poorly differentiated. IHC was scored for PDK1 (0-2), PTEN (0-1), and Stathmin (0-8), according to previously described scoring methods. Average IHC scores were calculated, and compared between cases of different grades.

Results: Our cohort contained 28 cases of conventional HNSCC; 2 cases that showed evidence of HPV association were excluded from analysis. We observed significantly higher expression of PDK1 in poorly differentiated tumors (average=1.64/2.00), compared to well- and moderately differentiated tumors (average=1.31/2.00). We also observed significantly higher expression of Stathmin in moderately and poorly differentiated tumors (average=5.24/8.00) compared to well-differentiated tumors (average=3.06/8.00). No significant differences in PTEN expression were observed.

Conclusions: Our results show that overexpression of PDK1 and Stathmin occurs in HNSCC. Our observations also indicate roles for these factors in progression of HNSCC, and suggest that increased Stathmin expression occurs earlier in tumorigenesis (i.e. distinguishes between well-differentiated tumors and higher-grade lesions), whereas increased PDK1 expression occurs later (i.e. distinguishes between poorly differentiated tumors and lower-grade lesions). These findings provide insight into the molecular mechanisms of this disease, and reflect analogous observations in other tumor types. Furthermore, these results suggest an adjunctive role for IHC in grading of HNSCC, of potential utility for equivocal cases and/or limited biopsy specimens. Overall, these observations provide further evidence of PI3K pathway activation in HNSCC tumorigenesis, and indicate this pathway's potential as a treatment target.

1362 Mammary Analog Secretory Carcinoma of the Salivary Gland; Distinction from Acinic Cell Carcinoma and Low-Grade Cribriform Cystadenocarcinoma by *ETV6*/*NTRK3* Fusion Gene Analysis and Immunohistochemical Study, Review of 18 Cases

M Urano, S Miyabe, T Nagao, Y Kiriyama, T Tsukamoto, Y Mizoguchi, M Kuroda. Fujita Health University, School of Medicine, Toyoake, Aichi, Japan; Aichi-Gakuin University, Nagoya, Aichi, Japan; Tokyo Medical University, Tokyo, Japan.

Background: Mammary analog secretory carcinoma (MASC) is a recently recognized salivary gland tumor with *ETV6* translocation similar to secretory carcinoma of the breast. Histologically, MASC quite mimics papillary-cystic and follicular type acinic cell carcinoma (AciCC) and low-grade cribriform cystadenocarcinoma (LGCCC). The aim of this study is to re-classify these tumors using *ETV6-NTRK3* fusion gene analysis and immunohistochemistry (IHC).

Design: We reviewed 18 cases which initially diagnosed as AciCC between 1997 and 2012. They were reevaluated by detailed histological pattern, detection of *ETV6-NTRK3* fusion gene by RT-PCR and FISH method, and IHC for CK CAM5.2, CK 34βE12, S-100, CK19, GCDFP-15, mammaglobin (MMG), MUC-1, α-amylase (AMY), p63, calponin, CD10, CK14, DOG1 and MIB-1.

Results: 18 cases were divided into two groups, as MASC (10 cases, M:F=6:4, Ave. 46 yrs) and AciCC (6 cases, M:F=3:3, Ave. 63 yrs), LGCCC (2 cases, M:F=0:2, Ave. 48 yrs) by *ETV6* translocation analysis. About 56 percent of the cases which formerly diagnosed as "AciCC" were re-classified as MASC. In IHC, vimentin, CK19, mammaglobin, MUC1, p63 and α-amylase were useful markers for distinction of these three histological types.

Summary of ETV6 fusion and IHC

	ETV6 fusion+(%)	vimentin+ (%)	CK19+(%)	MMG+(%)	MUC1+(%)	p63+(%)	AMY+(%)
MASC (n=10)	10/10 (100)	10/10 (100)	10/10 (100)	9/10 (90)	9/10 (90)	1/10 (10)	0/10 (0)
AciCC (n=6)	0/6 (0)	2/6 (33)	2/6 (33)	2/6 (33)	2/6 (33)	0/6 (0)	4/6 (67)
LGCCC (n=2)	0/ 2 (0)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)	0/2 (0)

Conclusions: Our data show a considerable incidence of MASC, and indicate that former "AciCC" cases would include virtual MASC cases. Molecular testing and proper IHC is recommended for an accurate differential diagnosis between MASC and current AciCC and/or LGCCC.

1363 Recurrent ARID1A-PRKD1 Fusion in the "Polymorphous Low Grade Adenocarcinoma/Cribriform Adenocarcinoma" Spectrum of Tumors

I Weinreb, L Zhang, L-C Chen, Y-S Sung, B Perez-Ordonez, A Skalova, SI Chiosea, RR Seethala, CR Antonescu. University Health Network, Toronto, ON, Canada; Memorial Sloan-Kettering Cancer Center, New York, NY; Charles University, Plzen, Czech Republic; University of Pittsburgh Medical Center, Pittsburgh, PA.

Background: Polymorphous low grade adenocarcinoma (PLGA) and cribriform adenocarcinoma of minor salivary gland (CAMSG) are low grade carcinomas that typically arise in the oral cavity and oropharynx, respectively. They appear to have a difference in behavior with CAMSG showing frequent metastases to the neck. Controversy exists as to whether these tumors represent separate entities or variants of one tumor spectrum. They appear to have significant overlap, but also have clinical/pathologic differences. Recently, several salivary carcinomas with recurrent fusions have been described; however there are no molecular studies to investigate this morphologic spectrum and controversial classification.

Design: Next generation RNA sequencing was performed on 1 candidate CAMSG and 2 classic PLGAs. Data was analyzed using FusionSeq, a modular computational tool developed to discover gene fusions from paired-end RNA-Seq data. Gene fusion candidates were validated by FISH, RT-PCR and Sanger sequencing. A total of 26 cases in this spectrum were reviewed, reclassified and tested by FISH for the potential fusion partners.

Results: Tumors were separated into 10 CAMSG, 9 classic PLGA and 7 with mixed/indeterminate features. A gene fusion involving ARID1A on chromosome 1 and PRKD1 on chromosome 14 was identified in the sequenced CAMSG case. The ARID1A-PRKD1 fusion was then validated by FISH and RT-PCR. A second case of CAMSG was found with rearrangement of both partner genes by FISH. One additional CAMSG case showed ARID1A rearrangement only (3/10, 30%). Two cases in the mixed/indeterminate category showed rearrangement of PRKD1 only (2/7, 29%). No cases of classic PLGA showed rearrangement of either gene (0/9). A total of 5/26 cases showed rearrangement of at least one of these genes (19%). The positive cases occured in base of tongue, floor of mouth, buccal, palate and parotid. No candidate fusion was found in the 2 sequenced classic PLGAs.

Conclusions: We describe a novel and recurrent ARID1A-PRKD1 fusion in cribriform adenocarcinoma of minor salivary gland. These findings represent the first molecular evidence of a difference from "classic" PLGA, however the positivity in mixed/indeterminate cases in this tumor spectrum warrants caution before completely excluding a shared pathogenesis for these tumor types.

1364 Basal Cell Adenocarcinoma and Basal Cell Adenoma of the Salivary Glands: A Clinicopathological Review of Seventy-Two Cases with Comparison of Morphologic and Immunohistochemical Features

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Background: Basal cell adenoma (BCA) and basal cell adenocarcinoma (BCAC), both represent salivary gland neoplasms with basaloid cell proliferation which can recur. We wished to correlate clinical outcome and morphologic features with growth and proliferation associated-markers between both entities to detemine if we could further refine indicators of behavior.

Design: We reviewed the clinical outcomes and pathologic features of 72 neoplasms diagnosed as BCA or BCAC gathered from our institution and consultative practice. Histologic observations included maximum mitotic activity and presence/absence of invasion into the surrounding normal tissues. Immunohistochemical studies for p53, Ki-67, caspase 3 and Bcl 2 were performed on archival material. Expression in >10% cells was considered overexpressed for p53. For Ki-67, at least 1000 cells were counted to establish a percentage. Caspase 3 was interpreted as number of cells staining per 10 high power fields, and Bcl 2 was categorized as positive with >5% staining.

Results: Followup was available in 69% of BCA (avg=67 months) and 59% of BCAC (avg=57 months). Establishing malignancy on the basis of invasion into the surrounding benign tissues, there were 43 BCA and 29 BCAC. 3/42 BCA recurred, one of which had a membranous pattern. 3/29 BCAC recurred with one of these patients dying with distant metastases. Overall BCAC showed a significantly higher mitotic rate, proliferation (Ki-67), apoptosis (caspase 3), and p53 expression and was more likely to lose Bcl2 expression compared to BCA. No strong correlation between growth control/proliferation markers and recurrence was noted in individual BCA's.

	BCA	BCAC
Average Mitoses per 10hpf	0.8 (0 to 5)	5.0 (1 to 20)
Average Ki-67 per 1000 cells	4.0% (0.1 to 10.5%)	15.5% (0.4 to 53.3%)
Average Caspase 3 per 10hpf	1 (0 to 9)	12 (0 to 37)
Cases Positive for p53	3/36 (8%)	8/18 (44%)
Cases with Loss of Bcl 2	1/36 (3%)	1/18 (6%)

Conclusions: BCA and BCAC are uncommon basaloid neoplasms with recurrence rates of 7.1% and 10.3%, respectively, in our series. Invasion into surrounding normal tissues by the BCAC did not help separate which cases recurred when compared to BCA although the one patient that died of disease did show this feature. Mitotic rates and expression of Ki-67, p53 and caspase 3 were all higher in BCAC than BCA but overlap between the results of these observations in individual cases does not allow for accurate prediction of outcome or recurrence alone.

1365 HOXC6 Overexpression as a Poor Prognostic Factor in Patients with Nasopharyngeal Carcinoma

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Background: Though the advances in diagnostic imaging and treatment modalities have achieved better locoregional control of nasopharyngeal carcinoma (NPC), it appears less satisfactory in final treatment outcomes. Through data mining from public domain, *homeobox C6* (*HOXC6*) was first identified as a differentially upregulated gene associated with regulation of transcription from RNA polymerase II promoter in the transcriptome of NPCs. HOXC6 belongs to a member of the homeobox family, deregulated expression of which has been observed in many tumor types including leukemia, breast, lung, and prostate cancer. Since its significant has not been systematically investigated in NPC, we therefore explored the significance of HOXC6 immunoexpression status and its association with cell proliferation index Ki-67 in a large cohort of NPC patients.

Design: HOXC6 and Ki-67 immunohistochemistry was retrospectively performed and analyzed using H-score method for biopsy specimens from 124 NPC patients who received standard treatment without distant metastasis at initial diagnosis. Those cases with H-score larger than the median value were defined as HOXC6 overexpression. The results were correlated with the clinicopathological variables, disease-specific survival (DSS) and metastasis-free survival (MeFS).

Results: HOXC6 overexpression was significantly positively associated with Ki-67 expression, and significantly associated with increments of tumor stage (p=0.024), advanced nodal status (p<0.001) and American Joint Committee on Cancer (AJCC) stage (p=0.002). Its overexpression also correlated with worse prognosis in terms of DSS (p=0.0008), MeFS (p=0.0047) univariately. In multivariate comparisons, HOXC6 overexpression still remained prognostically independent to portend worse DSS (p=0.015, hazard ratio=1.988) and MeFS (p=0.036, hazard ratio=1.899), together with advanced AJCC stages III-IV (p=0.024, DSS; p=0.043, MeFS).

Conclusions: HOXC6 expression is upregulated in a subset of NPCs and its increased immunoexpression significantly correlated with advanced stages and tumor aggressiveness, justifying the potentiality of HOXC6 as a prognostic biomarker and a novel therapeutic target of NPC.

1366 Use and Abuse of *In Situ* Hybridization for HPV in Head and Neck Tumors: Experience from a National Reference Laboratory

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Background: HPV related squamous cell carcinomas (SCC) of the head and neck (H&N) have a unique biology and improved response to treatment with favorable outcomes. Testing for biologically active HPV via DNA *in situ* hybridization (ISH) is widely used following diagnosis of H&N SCC for treatment and prognosis. The purpose of this study is to determine the use of HPV ISH testing in H&N tumors at a national reference laboratory.

Design: HPV ISH requests sent to our lab, which offers INFORM HPV III Family 16 Probe for high risk (HR) and INFORM HPV II Family 6 probe for low risk (LR) HPV (Ventana) were identified. Clients had the option to request LR, HR or both (Panel) HPV ISH. Information on the location of tissue, patient age and gender were provided by referring pathologists. H&E slides were independently reviewed by 3 pathologist, HPV ISH by one.

Results: Since we started offering Ventana HPV ISH (10/07), we have received 1045 requests for HR, 746 for LR and 2164 for Panel (HR+LR). At the time of abstract, 125 cases were reviewed. The concordance between 3 pathologists' malignant and

benign categories was 100%. 125/125 (100%) of cases had HR and 80/125 (64%) for LR HPV ISH testing done. Malignant diagnoses (n=74) included 55 keratinizing SCC (KSCC);14 non-keratinizing SCC (NKSCC); 3 papillary SCC (PSCC);1 undifferentiated carcinoma;1 SCC in situ (SCCIS). Benign diagnoses (n=51) included 37 squamous papilloma (SP);6 squamous hyperplasia (SH);3 normal epithelium (NE);2 laryngeal nodule (LN);1 sinonasal papilloma (SNP); and 1 each of condyloma and seborrheic keratosis (C/SebK) (last two are only samples outside of H&N (inguinal)). 74/74 (100%) of malignant cases had HR HPV ISH done (26/55 of KSCC, 9/14 NKSCC, 3/3 of PSCC, 0/1 of SCCIS, and 0/1 of undifferentiated carcinoma positive), 34/74 (46%) malignant cases had LR HPV ISH done (0/21 of KSCC, 2/11 of NKSCC, 0/1 of PSCC, 0/1 of undifferentiated carcinoma positive). 51/51 (100%) of benign cases had HR HPV ISH done (2/37 of SP, 1/6 of SH, 0/3 of NE, 0/1 of SNP, 0/2 of LN, 0/2 of C/SebK positive). 46/51 (90%) of benign diagnoses had LR HPV ISH ordered (16/34 of SP, 0/4 of SH, 0/2 of LN, 0/1 of SNP, 0/3 of NE, 1/2 of C/SebK positive).

Conclusions: Unnecessary HPV ISH testing in H&N lesions is common. 46% H&N carcinomas had LR HPV ISH and 100% of non-neoplastic and benign lesions had HR HPV ISH ordered. Our laboratory will discontinue offering HPV ISH Panel (HR+LR) in an attempt to reduce redundant testing.Training of clinicians and pathologists is essential in avoiding unnecessary costly testing.

1367 P16^{Ink4A}: No Correlation with Transcriptionally Active HPV16/18 or Outcomes in Oral Cavity Carcinoma

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Background: Transcriptionally active high-risk Human Papillomavirus (HR-HPV) is important in promoting oropharyngeal carcinomas (OPC) through binding of E6 and E7 viral oncoproteins with p53 and Rb tumor suppressor proteins, respectively, leading to inactivation. Loss of negative feedback secondary to Rb inactivation results in p16^{Ink4A} deregulation; p16^{Ink4A} overexpression is considered indicative of transcriptionally active and biologically relevant HPV infection. Recent studies suggest that p16^{Ink4A} overexpression alone, independent of HPV status, confers improved prognosis of OPC. Here we examine: (1) p16^{Ink4A} as a surrogate HPV biomarker in oral cavity carcinomas (OCC) and (2) whether p16^{Ink4A} and/or transcriptionally active HR-HPV can impact patient outcome for OCC.

Design: 151 patients with OCC were identified; patients with microinvasive carcinomas (≤ 4 mm) were excluded. RNA was extracted from archival specimens and reverse transcription was performed; residual DNA was removed by DNase digestion. Nested quantitative real-time PCR was performed with primers specific to HPV16 and HPV18 (E6/E7), respectively. Immunohistochemistry for p16^{Ink4A} expression was examined on whole tissue slides and OCC were classified as positive if strong, diffuse nuclear and cytoplasmic p16^{Ink4A} expression was seen (≥ +2 intensity,≥ 75% distribution). Data on demographics and outcome were collected. Fisher's exact test was used to analyze HPV status and demographics; Kaplan Meier curves were used to analyze HPV status and outcome.

Results:

	African Americans (n = 24)			Whites (n = 127)		
	HPV16 N = 3	HPV18 N = 2	Negative N = 19	HPV16 N = 33	HPV18 N = 10	Negative N= 84
P16 Overexpression (n = 84)	1/3	0/1	1/13	2/14	2/8	7/47
Average age	55.6			61.6		
Female: male	7.17			43.84		
T1/T2	14			98		
T3/T4	10			31		
N0	10			81		
N+	14			48		

HPV16+ OCC was seen in 36/151 (24%) cases, HPV18+ in 12/151 (8%) cases, and no double HPV16/18 infections were found. p16^{Ink4A} overexpression was demonstrated in 13/84 (15%) OCC, and did not correlate with either HPV16 or HPV18 status. Neither p16^{Ink4A} expression nor HPV status were significantly associated with overall survival, disease specific survival, or disease-free survival.

Conclusions: Our data suggests that, in contrast to OPC, p16^{Ink4A} overexpression is not a surrogate marker for transcriptionally active HPV in OCC. The lack of impact of p16^{Ink4A} overexpression with OCC outcome may be due to the small numbers of p16 ^{Ink4A} overexpressors found (15% or 13 patients).

1368 Analysis of the Anti-Tumor Effects of V-ATPase Inhibitor, Concanamycin A1, on Oral Squamous Cell Carcinoma

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Background: V-ATPase is involved in the acidification of the microenvironment around/in solid tumors, such as oral squamous cell carcinoma(OSCC). V-ATPase is thought to induce tumor invasion and multi-drug resistance in several malignant tumors. However, there is little information regarding the effects of V-ATPase inhibitors on OSCCs.

Design: We attempted to assess the effect of V-ATPase inhibitor, concanamycin A1 (CMA), on cell proliferation and apoptosis of OSCC cells (MISK81-5, SAS, HSC-4 and SQUU-B) *in vitro*. The effects of CMA on the cell viability and apoptosis were investigated by MTS assay and TUNEL staining, respectively. The mRNA and protein expression levels of apoptosis-related molecules after CMA treatment were analyzed by qRT-PCR and Western blotting, respectively.

Results: CMA treatment for 48 hr significantly suppressed the cell growth at low concentrations, and induced apoptosis in MISK81-5, SAS, and HSC-4 cells, but SQUU-B cells were highly resistant to CMA. Compared the expression of the pro- and anti-apoptotic factors in the SQUU-B cells with that in CMA-sensitive OSCC cells after treatment with CMA, whereas CMA activated p38, one of MAPK, in the CMA-sensitive OSCC cells, phosphorylation of p38 was not observed in SQUU-B cells. Moreover, CMA treatment induced comparative increase in Bcl-2 expression in the SQUU-B cells compared with that in the CMA-sensitive cells. However, when the SQUU-B cells were treated with CMA and a histone deacetylase inhibitor, suberoylanilide hydroxamic acid (SAHA), the SQUU-B cells became more susceptible to the CMA-induced apoptosis. SAHA treatment led to a significantly decreased Bcl-2 expression levels in comparison with that observed in the SQUU-B cells treated with CMA alone. Down-regulation of Bcl-2 partially induced decrease of the cell viability in the SQUU-B cells treated with CMA.

Conclusions: Our results indicate that CMA could have an anti-tumor effect on OSCCs and that the combination of CMA with SAHA may exhibit synergistic anti-cancer activity in certain CMA resistance cells.

1369 The Role of Alcohol-Regulated Long Non-Coding RNAs in the Pathogenesis of Oropharyngeal Cancer

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Background: Alcohol use is one of the primary risk factors for head and neck squamous cell carcinoma (HNSCC), yet the mechanism by which alcohol induces oropharyngeal cancer remains unclear. Although ethanol itself is not carcinogenic, acetaldehyde, or ethanal, the first metabolite of alcohol via the enzyme alcohol dehydrogenase, has been established as a suspected carcinogen. Long non-coding RNAs (lncRNAs) constitute a family of RNA longer than 200 nucleotides that do not code for proteins, but instead influence transcription factors associated with regulation of oncogenes, tumor suppressor proteins, self-renewal, and differentiation. This study sought to determine those key lncRNAs whose dysregulation by ethanol result in the acquisition of cancer stem cell properties in normal oral epithelial cells.

Design: Two normal oral keratinocyte cell lines (OKF4 and OKF6) were treated with various doses of 100% ethanol for 28 days to represent long-term alcohol use. The same cell lines were also treated with acetaldehyde for 72 hours to better investigate the *in vivo* mechanism of alcohol-induced HNSCC. Relative lncRNA and mRNA gene expression in ethanol treated cells were compared with that of parental cells through qPCR arrays.

Results: Comparison of relative lncRNA expression in ethanol treated cells with that of parental cells revealed that six lncRNAs (SAF, Zeb2Nat, Air, H19, IPW, and ncr-UPAR) are consistently upregulated. The expression of SAF and Zeb2Nat, two lncRNAs implicated in oncogenesis, exhibited the highest fold change, thus suggesting that SAF and Zeb2Nat are instrumental in the pathogenesis of alcohol-induced oropharyngeal cancers. The stem cell genes Nanog and Oct-4, and the epithelial-to-mesenchymal transition (EMT) gene Vimentin, were also upregulated through ethanol treatment, thereby suggesting that ethanol could promote expression of the EMT phenotype and stemness *in vivo*.

Conclusions: Ethanol exposure in normal oral epithelia dysregulates key lncRNAs previously implicated in cancer, and induces stem cell and EMT genes. Further investigation of the pathways of these lncRNAs may lead to prognostic indicators and therapeutic targets in treatment of alcohol-induced oropharyngeal cancers.

Hematopathology

1370 CXCR4 Expression Is Decreased on Plasma Cells from Patients with a Non-Hyperdiploid Karyotype

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Background: We have previously demonstrated that reduced CXCR4 expression is associated with a lymphoplasmacytoid immunophenotype and extramedullary disease in a mouse model of plasma cell myeloma (PCM). We and others have also shown that reduced CXCR4 is associated with poor survival in PCM patients, especially those treated with bortezomib. In this study we sought to optimize CXCR4 immunohistochemical staining of plasma cells in formalin fixed trephine core biopsies. In addition, we examined the association of CXCR4 expression with the cytogenetic and morphologic subtypes of PCM. We hypothesized that cases with a t(11;14) were more likely to have decreased/absent CXCR4 expression.

Design: We queried our cytogenetics database and identified 40 cases of PCM (including 24 diagnostic specimens) that harbored the most common recurrent cytogenetic abnormalities. We then reviewed bone marrow biopsies/smears and clinical data corresponding to the same accession dates. In addition, immunohistochemical (IHC) studies were performed on the core biopsies including CD20 and CXCR4. Stain intensity was scored from 0 (negative) to 3 (strongly positive). Statistical analysis was performed using Fisher's exact tests.

Results: 35% of PCM cases (14/40) were positive for surface expression of CXCR4, while nearly all control plasma cells were positive. Similar to previous studies, CD20 positivity was associated with the presence of t(11;14) ($P < 0.001$, Fisher's), but was not associated with strong or absent CXCR4 expression. Among the cases that bore a t(4;14) or t(14;16), all lacked CXCR4 expression ($P = 0.035$, Fisher's). In addition, among all cytogenetic abnormalities, cases without hyperdiploidy were more likely to lack CXCR4 ($P = .041$, Fisher's).

Conclusions: Our previous studies have demonstrated that bortezomib resistant PCM cells are more likely to have an immunophenotype intermediate between lymphocytes and plasma cells, including decreased CXCR4. We also have shown that reduced CXCR4 expression confers a worse prognosis in PCM patients. Our data confirm that PCM cells harboring a t(11;14) are more likely to express CD20 but do not appear to have