

**1159 Histopathologic Correlation Findings Associated with (Post-Hysterectomy) Vaginal Pap and HPV Test Results.**

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**Background:** High risk (hr) HPV infection is recognized as the dominant etiology for cervical carcinoma. Data on prevalence of hrHPV DNA in patients with abnormal vaginal Pap results and the subsequent histopathologic findings are very limited. This is the largest study to date correlating histopathologic follow-up findings associated with (Post-Hysterectomy) vaginal Pap and hrHPV test results.

**Design:** A computer-based search from our Copath files was conducted over a study period of 49 months between July 1, 2005 and July 30, 2009 to identify vaginal ThinPrep Pap test (TPPT) cytology cases reported as ASC-US, ASC-H, LSIL, or HSIL for which Hybrid Capture 2 (HC2) hrHPV DNA test results were also reported. Vaginal Pap and HPV test results and histopathologic follow-up outcomes were analyzed.

**Results:** During the study period there were 1320 vaginal Pap tests reported as ASC-US, ASC-H, LSIL, or HSIL which also had HPV testing. The prevalence of hrHPV infection in women with abnormal vaginal Paps is shown in Table 1. The average age of patients with vaginal ASC-US results was 56.5 (17-91), with ASC-H 58.9 (22-87), with LSIL 56.9 (27-92), and with HSIL 63.4 (42-93). 86 women with vaginal LSIL, ASC-H, HSIL cytology and hrHPV DNA testing had at least one follow-up biopsy. 373 women with vaginal ASC-US and HPV test results had cytologic and/or biopsy follow up results. The follow-up results are shown in Table 2.

HPV results associated with abnormal vaginal Paps

	#Case	#Positive	%
ASC-US	1125	244	22
ASC-H	36	21	58
LSIL	148	113	76
HSIL	11	9	82
Total	1320	387	29

	HPV Pos F/U#	VAIN2/3 (%)	VAIN1 (%)	HPV Neg F/U#	VAIN2/3 (%)	VAIN1 (%)
ASC-US	138	7 (5)	59 (43)	235	1 (0.4)	10 (4)
ASC-H	16	1 (6)	14 (88)	8	1 (13)	2 (25)
LSIL	48	7 (15)	34 (71)	11	0 (0)	7 (64)
HSIL	2	2 (100)	0	1	1 (100)	0 (0)
Total	204	17 (8)	107 (53)	255	3 (1)	19 (8)

**Conclusions:** The prevalence of hrHPV detection in abnormal vaginal Pap tests reporting ASC-US, ASC-H, LSIL and HSIL results are similar to those reported in abnormal cervical Pap test specimens from older women.

Histopathologic diagnoses of VAIN1 and VAIN2/3 were significantly increased following hrHPV positive abnormal vaginal Pap tests when compared to follow-up findings for patients with hrHPV negative abnormal vaginal Pap tests.

Reflex hrHPV DNA testing is a useful tool for assessing risk of histopathologic VAIN and in considering follow-up management options for women with abnormal vaginal Pap test results.

## Head & Neck

**1160 Rearrangement of the EWSR1 Gene Is a Consistent Feature in Hyalinizing Clear Cell Carcinoma of Salivary Gland.**

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**Background:** Hyalinizing clear cell carcinoma (HCCC) is a low grade salivary gland tumor with a characteristic nested and cordlike growth within hyalinized stroma. HCCC typically stains with squamous cell markers and shows occasional mucous cells making distinction from mucoepidermoid carcinoma (MEC) a challenge. We have observed that the characteristic clear cell nests of HCCC resemble those seen in soft tissue myoepithelial tumors (SMET). Up to 50% of MECs show a MAML2 gene rearrangement, while SMETs show EWSR1 rearrangement in 45% of cases. This has not been studied in HCCC to examine a possible link between these entities.

**Design:** 23 cases of HCCC with typical morphologic and immunohistochemical features were collected. FISH for EWSR1 and MAML2 were performed using custom BAC probes and 200 cells were scored per case. A case was called positive when  $\geq 20\%$  of cells had a break-apart signal.

**Results:** The 23 cases included 14 females & 9 males ranging from 25-87 yrs of age (avg. 59.4). Sites were 18 oral, 3 parotid, 1 nasal & 1 larynx. All cases showed nests and cords of clear cells in a hyalinized stroma. Follow up in 19 cases ranging from 2 to 195 mths (avg. 47.6) demonstrated 4 recurrences (21%). The remainder showed no evidence of disease (NED). There were no metastases or mortality in any case. Mucin was seen in 10 of 23 cases (44%) and varied from focal to diffuse. The tumors were positive for 34BE12 (16/17), p63 (19/20) and EMA (9/12). They were negative for S100, SMA and calponin. FISH showed a EWSR1 rearrangement in 18 of 22 cases (82%), while no MAML2 break-apart was detected in any of the cases (0/14), including all mucin containing tumors (0/7). No EWSR1 abnormality was present in any of the control cases tested, including 3 MEC with clear cells and 5 epithelial-myoepithelial carcinomas.

**Conclusions:** HCCC is a unique tumor entity that shares EWSR1 rearrangement with SMET, despite S100, SMA and calponin negativity. It is distinct from MEC, despite common mucinous differentiation. This critical distinction impacts on grading, since all mucin positive HCCC cases showed NED and would have been over graded (grade III) with conventional MEC grading schemes. FISH analysis for EWSR1 rearrangement can be used as a reliable tool when confronted with limited material or a challenging diagnosis.

**1161 Differentiated Dysplasia Is a Frequent Precursor or Associated Lesion in Invasive Squamous Cell Carcinoma (SCC) of the Oropharynx.**

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**Background:** The recognition of the spectrum of precursor lesions of oropharyngeal SCC, their classification and their grading have remained controversial over the last decades. This contrasts to vulvar cancer precursor lesions which are related to either HPV or chronic inflammation and can manifest – under the VIN paradigm (VIN I-III) or as differentiated dysplasia (dVIN), respectively. Oropharyngeal SCC precursor lesions are etiologically more variable, being related to smoking, HPV or chronic inflammation, and the spectrum of lesions may thus admittedly be wider, but still no clinically useful international consensus exists on histological types of precursor lesions and on the significance of individual types.

**Design:** We reviewed all available histological slides of patients with oropharyngeal biopsies (excluding the tonsil) and subsequent resection specimens with SCC on file in the archives of Department of Pathology of our hospital from 1992 until 2009.

**Results:** Five basic patterns of precursor lesions or SSC associated lesions were identified: **Pleomorphic** similar to full thickness severe laryngeal squamous dysplasia (24/155), **basaloid** stratified similar to anal basaloid dysplasia of AIN III (6/155), **differentiated** similar to dVIN or lichenoid lesions with large cells with large nuclei and prominent eosinophilic nucleoli, abundant eosinophilic cytoplasm and prominent desmosomes, with additional minimal basal cell layer or suprabasal cell irregularities (63/155), **mixed differentiating** pleomorphic with superficial maturation (43/155) as well as **verrucous** with open often raisin like nuclei without prominent nucleoli (11/155). Keratinization was a common but variable feature in differentiated, mixed differentiating and verrucous dysplasia. In 8/155 no precursor lesion could be identified. Progression of isolated differentiated dysplasia was documented in 13% of patients (21/155) over variable time periods ranging from months to years.

**Conclusions:** Full thickness epithelial dysplasia of either pleomorphic or basaloid type is present in only 20% of oropharyngeal SCC. Differentiated dysplasia is a frequent precursor or associated in situ lesion in oropharyngeal SCC. Failure to recognise differentiated dysplasia results in underdiagnosis of a sizable proportion of patients at risk for invasive carcinoma. Our cases of documented progression of differentiated dysplasia call for efforts to refine criteria for separation of differentiated dysplasia from morphologically related lichenoid lesions.

**1162 The Role of Postoperative Radiotherapy in the Management of Parotid Pleomorphic Salivary Adenomas: Is There Any Benefit?**

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**Background:** Introduction: Pleomorphic Salivary Adenoma is the most common tumour of the Parotid Gland. Currently no national management guidelines exist. The objective of this study was to evaluate the role of adjuvant radiotherapy.

**Design:** A retrospective study of all patients with a histological diagnosis of PSA between 1981 and 2008 in Greater Glasgow and Clyde was undertaken. From intra-operative notes and pathology reports, adherence to facial nerve, excision margins, capsule status and postoperative radiotherapy were analysed. Two cohort groups were identified. The first cohort underwent surgery alone while the second received postoperative radiotherapy. Post-operative recurrence, short and long-term complications were compared in the two groups.

**Results:** 201 patients were identified. 167 (83%) had surgery alone and 34 (17%) received adjuvant radiotherapy. Medical notes were retrievable in all patients receiving postoperative radiotherapy and in only 58 surgical patients. The rate of recurrence was 1.7% (1/58) in surgical patients and 2.9% (1/34) in patients receiving adjuvant radiotherapy. Short-term complications were significantly higher in the second cohort accounting for 100% compared to 38% in the first. While long-term complications 15/58 (25%) and 12/34 (32%) were observed in the first and second cohort respectively.

**Conclusions:** There was no significant difference in the recurrence rate between the two groups. Short term and long term complications were significantly higher in the postoperative radiotherapy cohort. Adjuvant radiotherapy is therefore not recommended in the treatment of PSA. As well as a higher long term complication rate, radiotherapy is less cost effective.

**1163 Perivascular Epithelioid Cell Neoplasms of the Head and Neck: Report of 3 Cases.**

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**Background:** Perivascular epithelioid cell tumors (PEComa) are a family of related mesenchymal tumors including angiomylipoma, lymphangiomyomatosis, lung clear cell sugar tumor and rare clear cell tumors in visceral and soft tissue sites. PEComas share overlapping histology, immunohistochemistry, and ultrastructure. They are characterized by a female preponderance, coexpression of melanocytic and smooth muscle markers and an intimate relationship with blood vessels. The growing interest in PEComas has led to increasing reports of these tumors in unusual locations. We describe a series of PEComas arising in the head and neck of 3 female patients and discuss the behavior of these distinctive tumors.

**Design:** H&E slides from 3 cases of head and neck PEComa from the consultation files of the authors were reviewed. All 3 cases were stained with melanocytic markers (HMB-45/Melan-A), S-100, muscle markers, vimentin, synaptophysin and pancytokeratin. Follow-up information was obtained from 2 cases.

**Results:** All patients were adult women with a wide age distribution (18, 26 and 71 years). 2 arose in the nasal cavity and 1 in the larynx. The signs and symptoms of PEComa of the larynx and the nasal cavity revealed no specificity and clinically the

patients presented with hoarseness and mass effect, respectively. None had enlarged cervical lymph nodes. The histopathological features observed in all 3 tumors were large polygonal-epithelioid cells with clear or eosinophilic granular cytoplasm and distributed in a perivascular pattern. The nuclei were eccentric with vesicular chromatin and small nucleoli. Some cells contained a brown cytoplasmic pigment resembling melanin or lipofuscin. Immunohistochemistry confirmed coexpression of melanocytic markers (HMB-45 or Melan-A) and muscle markers (actin or calponin) in all. No infiltrative growth, cytologic atypia, necrosis or mitotic activity was observed. All underwent complete surgical resection and 2 patients with follow-up are free of disease at 2 and 8 years.

**Conclusions:** We describe 3 cases of head and neck PEComa arising in the larynx and the nasal cavity. Similar to other reports of PEComas in unusual locations, these neoplasms appear to follow a benign clinical course. Familiarity with PEComas will ensure its consideration in the differential diagnosis of tumors of the head and neck with a similar morphology. Additional cases with a longer follow-up will help in understanding the pathogenesis and behavior of PEComas.

#### 1164 CTRC1/ MAML2 Fusion Transcript in Central Mucoepidermoid Carcinoma of Mandible – Diagnostic and Histogenetic Implications.

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**Background:** Intraosseous salivary gland carcinomas are extremely rare, comprising only 2–3% of all mucoepidermoid carcinomas (MECs) reported. The t(11;19) translocation and its CRTCI/MAML1 fusion transcript have been identified in MEC at different sites and are believed to be associated with the development of a subset of these tumors. However, the status of the fusion transcript has not been reported in intraosseous MEC.

**Design:** Here, we report three examples of central MEC of the mandible, including a case with a history of primary retromolar MEC. Reverse transcriptase–polymerase chain reaction (RT-PCR) and DNA sequencing analyses of the microdissected components of these tumors were used for detection and verification of the fusion transcript.

**Results:** We identified, for the first time, the t(11;19) fusion gene transcript in central MEC, including in the previous primary retromolar MEC. No fusion transcript was detected in the second primary non-central MEC or in another central MEC. The results indicate that central MEC can manifest the fusion transcript.

**Conclusions:** Our findings show that central MEC of the mandible can manifest the fusion transcript. In case 3, the lack of the fusion transcript in the primary non-central MEC indicates an independent central second primary tumor. Since the initial clinical and radiological diagnosis in two central low-grade MECs was a benign odontogenic cyst, our findings support a future role for the fusion analysis in initial diagnostic efforts. In summary, we have identified, to our knowledge for the first time, t(11;19) fusion gene transcripts in a subset of central MEC, originating from ectopic salivary rests, and the absence of the fusion transcript in MEC with glandular odontogenic precursors. This finding may have diagnostic and histogenetic roles in the future analysis of this entity.

#### 1165 CpG Island Methylation Profiling in Human Salivary Gland Adenoid Cystic Carcinoma.

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**Background:** A better understanding of key molecular changes during the pathogenesis of salivary gland adenoid cystic carcinoma (ACC) could impact strategies to reduce recurrence and mortality from this cancer. Two epigenetic events involved in the pathogenesis of cancer are hypermethylation of tumor-suppressor gene promoters associated with transcriptional repression and hypomethylation associated with gene reexpression possibly linked to oncogene activation and genomic instability. The aim of this study was to identify genes (1) strongly deregulated by epigenetic CpG island methylation, (2) involved in development and survival of ACC.

**Design:** We analyzed 16 patients (tumor ACC and matching normal tissue) for aberrant hypermethylation using the methylated CpG island amplification and microarray (MCAM) method. For validation, CpG island regions of four genes, showing high changes in methylation with the MCAM method, were analyzed by Pyrosequencing.

**Results:** MCAM results for hyper- and hypomethylation show variable log 2 ratios between ACC and normal tissue: 1.3 to 2.3 fold increase in hypermethylation for 32 associated genes, and 1.3 to 1.5 fold changes in hypomethylation for seven associated genes. Strongest hypomethylations occur in gene regions near FBXO17 (catabolism, ubiquitination), PHKG1 (catabolism, phosphorylation), and cell surface proteins LOXL1, DOCK1 and PARVG. Strongest hypermethylation occurs in regions around TSS of genes encoding predominantly transcription factors (EN1, FOXE1, GBX2, FOXL2, TBX4, MEIS1, Lbx2, NR2F2, POU3F3, IRX3, TFAP2C, NKX2-4, PITX1, NKX2-5), except for 13 other genes with different functions (MT1H, EPHX3, AQPEP, BCL2L11, SLC35D3, S1PR5, PNLIPRP1, CLIC6, RASAL, XRN2, GSTM5, FNDC1, INSR). Four CpG island regions, showing significant hypermethylation in MCAM, and associated with EN1, FOXE1, TBX4, and PITX1 were validated by Pyrosequencing. This method supported the MCAM results, with an average difference of 50% in CpG hypermethylation over all 4 gene regions investigated.

**Conclusions:** The genes associated with the highest CpG island hypermethylation in neoplastic tissue imply, that these genes are actively expressed in normal tissue and silenced in salivary gland ACC. To our knowledge, this is the first time that these genes have been implicated in salivary gland tumorigenesis.

#### 1166 A Comparative Analysis of LEF-1 in Odontogenic and Salivary Tumors.

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**Background:** Odontogenic tumors may occasionally prove difficult to distinguish from salivary gland tumors in the oral cavity, particularly those with basaloid morphology. We evaluate the potential utility of LEF-1, a nuclear transcription factor involved in tooth and hair follicle development, in distinguishing between these tumors.

**Design:** Immunohistochemical staining for LEF-1 (sc-8591, 1:200 dilution; Santa Cruz Biotechnology, Santa Cruz, CA, USA) was performed on paraffin sections from 23 basaloid salivary gland carcinomas (16 basal cell adenocarcinomas (BCAC), 7 adenoid cystic carcinomas (ACC)), and 25 odontogenic tumors (13 ameloblastomas, 5 calcifying cystic odontogenic tumors, 3 adenomatoid odontogenic tumors, 2 calcifying epithelial odontogenic tumors, 1 ameloblastic carcinoma, and 1 squamous odontogenic tumor). Hair follicle bulb was used as a positive control.

Nuclear staining intensity (grades 0-3) and percentage of positive cells were recorded. A value between 1 and 4 was assigned for the percentage of positive cells (1 for 0-25%, 2 for 26-50%, 3 for 51-75% and 4 for 76 to 100%). The product of intensity and positive cells was calculated for a composite score (range: 0-12). Positivity was defined as at least 2+ intensity in >50% of tumor cells which required a composite score of >6.

**Results:** LEF-1 was positive in 80% (4/5) calcifying cystic odontogenic tumors, 15% (2/13) of ameloblastomas, and 33% (1/3) of adenomatoid odontogenic tumors. The calcifying epithelial odontogenic tumors, ameloblastic carcinoma, and squamous odontogenic tumor were negative.

Strong and diffuse LEF-1 expression was seen in 69% (11/16) of BCACs, which was significantly more frequent than in ACCs, which were only positive in 14% (1/7) of cases (Fisher exact, 2-tail  $p = 0.027$ ). No LEF-1 staining was seen in any components of normal salivary gland parenchyma. LEF-1 expression was greatest in the peripheral basal layer of BCACs. 100% (4/4) of tubulotrabeccular and (1/1) cribriform patterned BCACs stained for LEF-1; whereas, 67% (4/6) of solid and 40% (2/5) of membranous patterned expressed LEF-1.

**Conclusions:** We document for the first time the presence of LEF-1 expression in select basaloid salivary gland carcinomas. This finding combined with the low frequency of immunoreactivity in most of the tested odontogenic tumors argues against its utility in distinguishing salivary gland tumors from odontogenic tumors. However, it appears that BCACs frequently express LEF-1 suggesting that these are truly ‘dermal analogue tumors’ phenotypically. This strong expression may have utility in distinguishing these tumors from ACC.

#### 1167 Does pTis Exist for HPV Driven Tonsillar Carcinoma?

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**Background:** Many tonsillar primary tumors (~13%) present clinically as neck nodal metastases. Indolent tonsillar primaries may be found upon histologic examination. In HPV-driven tumors, positivity for HPV in the nodal met may serve to guide the surgeon to the tonsil. HPV-driven tonsillar carcinoma begins in the reticulated crypt epithelium, possibly through viral integration. P16 is a putative surrogate of HPV infected cells, although not synonymous with neoplasia. The crypt serves an immune function through the presentation of antigen via specialized squamous cells, “M” cells that endocytose antigens at their apical membrane and present them to B and T lymphocytes and other immune cells. The basement membrane is not complete in the reticulated crypt epithelium, which may enhance the immune function. The overlay of epithelial cells and immune cells makes this a difficult part of the tonsil to examine, and the distinction of carcinoma in-situ from invasion may be indistinguishable. We examined the reticulated crypt epithelium in normal and neoplastic tonsils to further investigate whether a carcinoma is ever really “contained” by basement membrane if arising in a tonsillar crypt, as in HPV-driven tonsillar tumors.

**Design:** Tonsils from 3 cases of cTXN1 were reviewed. IHC for p16, collagen IV and PAS stain were examined on one case. The tonsils showed small crypt centered carcinomas positive for HPV 16 by a two-tiered approach, using the Roche Linear Array HPV Genotyping Test® kit. Normal tonsil was also examined by transmission electron microscopy (EM).

**Results:** The small tumor s appeared to partially “line” the crypts and focally extend to the surface epithelium. P16 stained the tumor cells and showed a highly irregular epithelial-lymphoid interface characteristic of the specialized crypts. Lack of BM stain was greatest in the H&E areas of greatest irregularity. The EM confirmed an apparent basement membrane of the surface squamous epithelium with loss and disruptions in the continuity within the crypts, associated with many small blood vessels.

**Conclusions:** H&E examination of tonsillar crypt primary tumors, such as the HPV-driven family of tumors, does not allow the observer to make a distinction of in-situ carcinoma from invasive carcinoma because this distinction does not appear to exist. Even early onset neoplasia appears to be already invasive into the tonsillar stroma with metastatic potential. The stage of pTis, likely seldom used, may therefore need to be excluded from the future system of staging for tonsillar crypt tumors

#### 1168 Detection of Human Papilloma Virus and P16 Expression in High Grade Adenoid Cystic Carcinoma of the Head and Neck.

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**Background:** Squamous cell carcinoma (SCC) of the head and neck, particularly non-keratinizing squamous cell carcinoma and basaloid squamous cell carcinoma (BSCC), may be difficult to distinguish from high grade adenoid cystic carcinoma (HGACC). Oropharyngeal SCC is frequently associated with human papilloma virus (HPV), especially HPV 16, which is thought to cause p16 overexpression by functional

inactivation of the retinoblastoma protein. As such, p16 is widely considered a surrogate marker for HPV infection. However, the incidence and relevance of p16 expression and HPV infection in HGACC of the head and neck is unknown.

**Design:** The Mayo Clinic pathology database was searched for cases of HGACC of the head and neck in which resection specimens with paraffin blocks were available. Twelve cases were identified (6 submandibular gland, 4 parotid gland, sublingual gland, nasal cavity). All cases were tested by immunohistochemistry for p16 expression and *in situ* hybridization (ISH) for high and low risk HPV. Clinical histories were obtained from existing medical records.

**Results:** All study cases exhibited focal p16 immunoreactivity (100%). Of the 12 cases, only one (nasal) was positive for HPV by ISH (8%).

**Conclusions:** These findings suggest that the presence of HPV infection in HGACC is infrequent, even in the presence of p16 overexpression. Nevertheless, HPV positivity should not be used to exclude the possibility of HGACC when the differential diagnosis includes both BSCC and HGACC. Moreover, although p16 overexpression is often used as a surrogate marker for HPV infection in SCC, it cannot be used in this manner in the setting of HGACC.

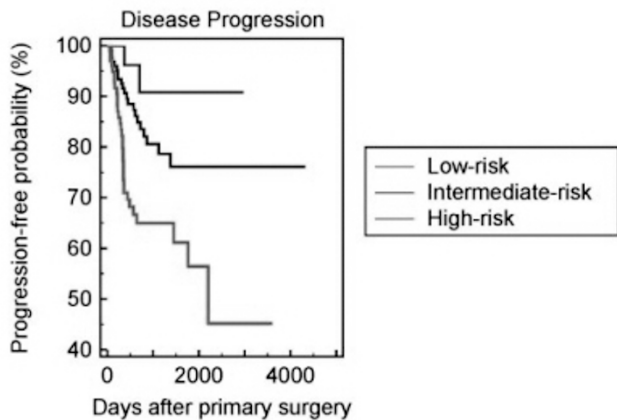
#### 1169 Risk Categories Predicts Disease Progression and Overall Survival for Low Stage Oral/Oropharyngeal Cancers.

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**Background:** Our validated Risk Model predicts outcome for patients with oral/oropharyngeal squamous cell carcinomas (SCC). A risk score is assigned by assessing tumor worst pattern of invasion (WPOI) & lymphocytic host response (LHR) at the tumor interface, and perineural invasion. Identifying low-stage cancer patients at high-risk for disease progression (DP) could become the basis for developing new treatment protocols. We test the hypothesis that the Risk Model has added prognostic value *within* a low-stage patient cohort.

**Design:** This multi-institutional observational study was limited to patients with Stage I/II oral/oropharyngeal SCC treated by primary surgery +/- radiation. Resection specimen slides were reviewed by MBG, blinded to outcome, and the risk category assigned. The resection margin standard was 5 mm. Outcome was classified as disease progression (DP), overall survival (OS), and disease-specific survival. Univariate and multivariate Cox regression analyses were performed and Kaplan Meier survival curves were generated for these outcomes, and these variables: WPOI, LHR, PNI, score, risk category, stage, tumor site, margins, center, age, sex, and treatment. Significance was set at  $p \leq 0.05$ .

**Results:** This cohort is comprised of 172 men, 113 women, from 7 centers, ages 23-95, 168 Stage I, 117 Stage II, 241 oral and 44 oropharyngeal tumors; 74% of patients were treated by surgery alone, the rest received adjuvant radiotherapy. The mean followup interval was 32 months. Disease-progression and disease-specific mortality rates among Stage I and Stage II patients were 19%, 10%, 20%, and 8%, respectively. Regression analysis show that the risk categories significantly associate with decreased time to DP ( $p = 0.0104$ , HR 1.95, 95% CI 1.17, 3.24) and OS, ( $p = 0.0156$ , HR 1.70, 95% CI 1.11, 2.61) when adjusted for confounders.



**Conclusions:** We demonstrate that the Risk Model has added prognostic value for low-stage patients with respect to DP and OS. These data justify the need for future randomized clinical trials offering adjuvant radiotherapy for the sole indication of high-risk status.

#### 1170 Examination of MYB in Adenoid Cystic Carcinoma and Other Neoplasms of the Head & Neck.

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**Background:** Adenoid cystic carcinomas (ACC) are characterized by a recurrent and tumor type-specific t(6;9)(q22-23;p23-24) chromosomal translocation. Recent studies have shown that this translocation results in a novel fusion of the MYB oncogene with the transcription factor gene NFIB in ACC arising in several anatomic sites including the salivary glands. In this study of ACC and other neoplasms of the head & neck, we explored the diagnostic utility of an antibody that recognizes residues near the N-terminus of MYB and compared the results with the expression of MYB-NFIB fusion transcripts.

**Design:** We immunostained 68 cases of ACC of various anatomic sites and 113 non-ACC tumors of the head and neck. An anti-MYB rabbit monoclonal antibody (clone EP769Y, 1:200 dilution, Epitomics, Inc.) was used after pressure cooker antigen retrieval. MYB immunostaining was considered positive if greater than 5% of tumor cells displayed strong nuclear staining. RT-PCR and nucleotide sequence analysis were used to determine the MYB-NFIB gene fusion status, while quantitative real-time PCR was employed to examine MYB expression levels.

**Results:** 53 ACC arose in the aerodigestive tract, while 15 were from other sites. 82% of ACC were positive for MYB compared with 14% of non-ACC neoplasms. The sensitivity and specificity of MYB immunostaining for ACC was 82% and 86%, respectively. There was no difference in staining according to the grade of ACC, but a peculiar zonal pattern of staining was observed in various cases. Approximately 70% of a subset of ACCs (n=30) were fusion-positive and these also were consistently positive by IHC. Non-ACC tumors that stained positive for MYB were typically fusion-negative. Analysis of the expression levels of MYB by quantitative real-time PCR showed that 90% of the fusion-positive cases also expressed high levels of MYB transcripts.

**Conclusions:** Fusion-positive ACC typically express high levels of MYB transcripts and are positive for MYB with IHC. MYB immunostaining is sensitive and specific for the diagnosis of ACC, but some non-ACC neoplasms in the differential diagnosis are also labeled. The zonal pattern of positive and negative staining observed in some cases may relate to issues of formalin fixation and relatively brief half-life of the protein.

#### 1171 Practical Clinical Application of a Histologic Risk Assessment Model for Head and Neck Squamous Cell Carcinoma.

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**Background:** A very promising histologic risk assessment model for head and neck squamous cell carcinoma (HNSCC) has been proposed in which a risk score is derived from the sum of scores for infiltrative pattern, perineural invasion, and lymphocytic response (Brandwein-Gensler *et al.* Am J Surg Pathol 2005;29:167-178). Risk assessment model validation included live training sessions for the pathologists who applied the model (Brandwein-Gensler *et al.* Am J Surg Pathol 2010;34:676-688). We questioned whether interobserver agreement would differ if observers applied the model after reading the articles cited above without the benefit of live training sessions, more closely simulating how the model would most likely be implemented in clinical practice.

**Design:** 6 pathologists and 4 residents at our institution applied the model to 20 cases of HNSCC from our files after learning the scoring method in the model by reading the articles cited above. No training sessions were given. Interobserver agreement was measured using the scores that the observers assigned.

**Results:**

Interobserver Agreement (as expressed by Kendall's Coefficients of Concordance)

Observers	Infiltrative Pattern	Perineural Invasion	Lymphocytic Response	Risk Score
Pathologists	0.46	0.52	0.63	0.68
Residents	0.43	0.60	0.63	0.71
Pathologists + Residents	0.38	0.49	0.59	0.66

Observers reached complete agreement on risk classification (low/intermediate risk vs. high risk) in 7 of the 20 cases.

**Conclusions:** In our study, interobserver agreement showed some variation across the risk score elements in this HNSCC risk assessment model. Pathologist, resident, and overall risk score correlation coefficients obtained without training sessions were similar (0.68, 0.71, and 0.66, respectively). Prior to widespread implementation of the model, evidence of repeated achievements of greater levels of interobserver agreement would be highly desirable. The establishment of an Internet-based, annotated image bank may provide an easily-accessible training resource that may lead to a successful, broad incorporation of the model into clinical practice.

#### 1172 Muscle Invasion in Oral Tongue Carcinoma as a Predictor of Nodal Status and Local Recurrence: Just as Effective as Depth of Invasion?

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**Background:** The most important prognostic factor in patients with early head and neck squamous cell carcinoma (SCC) is the presence of disease in cervical lymph nodes. Depth of tumor invasion (DOI) is a histologic feature that consistently correlates with lymph node metastasis and since 2000, the CAP has recommended that DOI be included in pathology reports; however, there are difficulties with accurately assessing and utilizing depth of invasion. The method is fraught with inconsistencies such as where to take the measurement and what should be used as the appropriate cut-off. These difficulties are magnified in frozen sections where freeze artifact can make accurate measurement even more troublesome. Conversely, identifying tumor at or within muscle could be achieved with minimal difficulty. The aim of this study is to identify a simpler and more reproducible method of determining and reporting DOI by using skeletal muscle invasion as a surrogate marker of depth.

**Design:** After IRB approval, oral tongue SCC AJCC stage T1 cases were identified in the Emory Department of Pathology database. The patient clinical histories were reviewed via electronic records. 61 cases had a minimum of 2 years of clinical follow-up and were included in the study. The cases were then examined histologically to assess skeletal muscle invasion, perineural invasion, and greatest depth of invasion. The two methods of measurement were analyzed to determine the positive predictive value (PPV) of DOI or muscle invasion with both nodal disease and local recurrence. The PPV between depth of invasion and muscle invasion were compared.

**Results:**

Muscle Invasion Vs. Nodal Status

	Muscle Invasion Present	Muscle Invasion Absent	
Positive Lymph Nodes	10	1	PPV=23.3%
Negative Lymph Nodes	33	17	

Depth of Invasion VS. Nodal Status

	Depth ≥ 3	Depth < 3	
Positive Lymph Nodes	11	0	PPV=29.7%
Negative Lymph Nodes	26	24	

**Conclusions:** Although the PPV of muscle invasion in regards to nodal status was slightly less than DOI, it represents a more easily reproducible parameter that could serve to guide the surgeons, especially in the frozen section room in determining if the case warrants an elective neck dissection in a cN0 neck. This method would also minimize inaccurate measurement due to tissue shrinkage from formalin fixation, as well as the inherent difficulties with taking measurements. Interestingly, the PPV of local recurrence was higher with muscle invasion than DOI (data not shown), and may represent an important indicator for extent of resection.

### 1173 Equivocal p16 Immunostaining in Head & Neck Carcinoma: Staining Pattern May Determine HPV Status.

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**Background:** P16 immunohistochemistry (IHC) is commonly used as a surrogate marker for human papilloma virus (HPV) detection in squamous cell carcinomas of the head and neck (SCCHN), especially in the tonsil and tongue base. However, the HPV status of tumors not staining strongly/diffusely for p16 is difficult to interpret. These cases may require the use of PCR, a costlier procedure than IHC, as a final arbiter of HPV status. We aim to determine if certain staining patterns or tumor characteristics in cases of equivocal p16 staining are predictive of PCR HPV status.

**Design:** A retrospective review was performed on pathology reports of all SCCHN that underwent p16 IHC and PCR with a linear array HPV genotyping test kit (Roche Diagnostics, Laval, Canada) in our institution. Each report with equivocal p16 IHC results (i.e. focal, weak, inconclusive or equivocal staining) was compared to the final PCR report. Keywords describing the staining pattern in the p16 IHC report in addition to tumor characteristics such as age, sex, site and diagnosis were compared to the final PCR HPV status. Statistical analysis was performed using the  $\chi^2$  test.

**Results:** Thirty-one of 92 (34%) SCCHN that underwent PCR HPV testing from 2007-2010 had equivocal p16 IHC results. Of these, 13 (42%) tested positive and 18 (58%) negative for HPV by PCR. Mean age was 61.5 (range 43-70) with a male/female ratio of 2.1. Specimen sites included tongue, oral cavity mucosa, larynx, tonsil and neck lymph node. Diagnoses included conventional SCC, non-keratinizing SCC and SCC in-situ. Comparing age, sex, tumor site and diagnosis to HPV PCR status showed no statistically significant findings. However, comparing IHC staining keywords to HPV status was statistically significant ( $\chi^2=22.1$ ;  $p=0.0002$ ) with isolated staining strongly associated with negative HPV status and equivocal associated with positive HPV status.

Distribution of HPV PCR Results Based on IHC Keywords

Staining pattern keyword	No. of cases with HPV+ PCR	No. of cases with HPV- PCR
Isolated	0	11
Focal	2	6
Focal & weak	3	0
Weak	2	1
Equivocal	6	0

n=31; p=0.0002

**Conclusions:** Our results suggests that the presence of isolated cells staining for p16 in SCCHN is not associated with the presence of HPV by PCR and that PCR testing may not be necessary to determine HPV status in these tumors. Nonetheless, there is still a significant group of tumors with ill-defined "equivocal" or "weak" p16 staining patterns that requires PCR or in-situ hybridization to conclusively determine HPV status.

### 1174 DOG1: A Novel Marker of Salivary Acinar Differentiation.

J Chenevert, U Duvvuri, JH Kim, RR Seethala. University of Pittsburgh Medical Center, PA.

**Background:** DOG1 (TMEM16A) is a calcium-activated chloride channel expressed in a variety of normal and tumor tissues, most notably gastrointestinal stromal tumors. In murine models, DOG1 is highly expressed in salivary gland acini and noted to be critical for saliva secretion. We herein validate this finding in human salivary tissues and describe its distribution in normal salivary tissues and tumors.

**Design:** TMEM16A mRNA levels were evaluated by quantitative RT-PCR in 10 normal parotid and 10 normal squamous epithelial frozen tissue samples. Immunohistochemical staining for DOG1 (Clone 1.1, Zeta Co, Sierra Madre, CA, dilution: 1:50) was then performed on paraffin sections from 30 parotid tumors (25 with adjacent normal) as well as 3 submandibular sialadenitis cases and one sinonasal mucoserous gland biopsy. Tumors consisted of 18 acinic cell carcinomas (AcICC) and 12 non AcICC (6 salivary duct carcinomas, 4 pleomorphic adenomas, 1 Warthin tumor, and 1 sebaceous lymphadenoma). Immunohistochemical parameters evaluated included: cell type distribution, % of cells, intensity (scale: 0-3) and subcellular localization.

**Results:** TMEM16A mRNA levels were significantly higher (5-fold increase,  $p<0.05$ ) in normal parotid tissue as compared to squamous epithelium. Immunohistochemical evaluation for DOG1 demonstrated diffuse 2+ apical (luminal) staining in normal serous acini in all non neoplastic salivary tissue types, 1+ apical staining in mucous acini, and very focal 1+ staining of intercalated ducts. Myoepithelial cells, striated and excretory ducts were all invariably negative. In tumors, DOG1 staining was almost entirely restricted to AcICC. All 18 cases contained 3+ apical staining with 16 cases showing diffuse staining (100% of cells). Truly solid areas showed far less apical staining, while

areas with mainly vacuolated cells had only focal 1-2+ staining compared to other cell types in AcICC. 15/18 cases showed cytoplasmic staining 1-2+ and 10/18 cases showed membranous staining 1-2+. All the non-AcICC tested so far are largely negative, though 1+ apical staining was noted in scattered ducts of pleomorphic adenoma.

**Conclusions:** DOG1 is highly expressed in human salivary acini. It is retained in AcICC and can potentially serve as a marker of acinar differentiation. A preliminary survey of non-AcICC supports the specificity of DOG1 though this must be validated.

### 1175 Absence of Merkel Cell Polyomavirus in Primary Parotid High Grade Neuroendocrine Carcinomas.

RD Chernock, EJ Duncavage, DR Gnepp, SK El-Mofty, JS Lewis. Washington University School of Medicine, St. Louis, MO; University of Utah, Salt Lake City; Alpert Medical School at Brown University, Providence, RI.

**Background:** High grade neuroendocrine carcinoma of the parotid gland is a rare malignancy that may cause diagnostic confusion with metastatic neuroendocrine (Merkel cell) carcinoma of the skin, which often occurs on the head and neck, and may metastasize to parotid lymph nodes. Immunohistochemistry of these two tumors overlaps, as a subset of primary parotid gland neuroendocrine carcinomas demonstrate a "Merkel cell" immunophenotype [cytokeratin 20 (CK20) dot-like positivity]. Thus, additional diagnostic tools to differentiate these two tumor types may be clinically useful. Cutaneous Merkel cell carcinoma is known to harbor Merkel cell polyomavirus (MCPyV) in up to 80% of cases. However, the presence or absence of MCPyV in parotid neuroendocrine carcinomas has not been investigated.

**Design:** High grade parotid gland neuroendocrine carcinomas in patients who had no clinical evidence of primary skin Merkel cell carcinoma, lung or other neuroendocrine carcinomas were included in the study. Synaptophysin and/or chromogranin as well as CK20 immunohistochemistry results, performed as part of the original diagnostic evaluation in all cases, were recorded. The presence of MCPyV was evaluated by both immunohistochemistry (CM2B4 clone) and real-time PCR directed against the proximal large T (LT) antigen. Cases of Merkel cell carcinoma of the skin were used as positive controls.

**Results:** Seven cases were identified. All were either chromogranin and/or synaptophysin positive. Six of the seven cases were chromogranin positive and five were synaptophysin positive. Microscopically, five had small cell and two had large cell morphology. Four of the five small cell carcinomas (80%) were CK20 positive. One of the two large cell carcinomas (50%) was CK20 positive. All but one case had cervical lymph node metastases. MCPyV LT was not detected in any of the seven tumors, either by immunohistochemistry or PCR with adequate controls.

**Conclusions:** Primary parotid gland high grade neuroendocrine carcinoma, like Merkel cell carcinoma of the skin, is frequently CK20 positive. However, MCPyV LT was not detected in any of these tumors. Thus, despite similar morphologic findings these two tumors likely arise from different pathways. Furthermore, viral testing may aid in distinguishing primary tumors from metastatic Merkel cell carcinoma in the parotid gland, as a positive result would favor a metastasis.

### 1176 Detection and Significance of Human Papillomavirus, p16 and p21 Overexpression in Squamous Cell Carcinoma of the Larynx.

RD Chernock, JS Lewis, Q Zhang, WL Thorstad, SK El-Mofty. Washington University, St. Louis, MO.

**Background:** Numerous studies have shown a strong etiologic relationship between human papillomavirus (HPV) and the majority of oropharyngeal squamous cell carcinomas (SCCs). HPV-positive SCC of the oropharynx has become increasingly recognized as a distinct variant of head and neck SCC characterized by better patient outcome, nonkeratinizing histology and overexpression of p16. More recently, overexpression of p21, a member of p53 cascade, has also been described as a feature of these tumors. The role of HPV in tumorigenesis of laryngeal SCC and its clinical implications has not been adequately investigated. The aim of this study is first, to identify the presence of HPV and the associated changes in expression of p16 and p21 in laryngeal SCC, and second to determine the effects of these alterations on patient outcome.

**Design:** Cases of laryngeal SCC from 1997 to 2007 with primary surgical pathology material for review were retrieved from a radiation oncology database. All patients received either upfront surgery with postoperative radiation or definitive radiation based therapy. Immunohistochemistry for p16 and p21 as well as HPV *in situ* hybridization (ISH) were performed. PCR for HPV was performed on p16 positive tumors.

**Results:** Of 76 laryngeal SCCs, none showed nonkeratinizing histology. p16 was positive in 21 (27.6%) and p21 was positive in 34 (44.7%) of the tumors. p16 and p21 status strongly correlated with each other ( $p=0.0038$ ). Only two tumors were HPV positive by ISH (both p16 and p21 positive cases). Material was available for HPV PCR in 20 of the 21 p16 positive cases. Of these, 13 (65%) were HPV positive by PCR, all of which were high risk genotypes. Patients with p21 positive tumors had better overall survival (69% at 3 years) than those with p21 negative tumors (51.0% at 3 years) [ $p=0.045$ ]. There was also a strong trend towards better overall survival in the p16 positive group ( $p=0.058$ ).

**Conclusions:** A subset of laryngeal SCCs display a molecular profile typical of HPV-positive SCC of the oropharynx (p16 and p21 overexpression). These tumors lacked the nonkeratinizing histologic features characteristic of HPV-positive oropharyngeal SCC but were frequently HPV-positive by PCR. They also appear to be associated with better overall patient survival.

### 1177 Sentinel Lymph Node Strategy with Serial Sections with Immunohistochemistry in T1-T2N0 Oral and Oropharyngeal Cancers Leads to 30% Upstaged Patients.

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**Background:** The aim of this study was to evaluate the lack of accuracy in neck staging with the classical technique (i.e., neck dissection and routine histopathology) with the sentinel node (SN) biopsy in oral and oropharyngeal T1-T2N0 cancer.

**Design:** Cross-sectional study with planned data collection was used. In 50 consecutive patients, the pathological stage of sentinel node (pSN) was established after analyzing SN biopsies (n = 148) using serial sectioning (5 microns thick sections every 250 microns) and immunohistochemistry with AE1/AE3. Systematic selective neck dissection was performed. The pN stage was established with routine histopathologic analysis of both the non-SN (n = 1075) and the 148 SN biopsies.

**Results:** The sensitivity and negative predictive value of pSN staging were 100 percent. Conversely, if one considers pSN staging procedure as the reference test for micro- and macro-metastasis diagnosis, the sensitivity of the classical pN staging procedure was 50 percent (9/1; 95% CI 26.9-73.1) and its negative predictive value was 78 percent (95% CI 61.9-88.8). Nine pN0 patients were pSN positive.

comparison of pN and pSN staging method

	pN+	pN0	total
pSN+	9 (18%)	9 (18%)	18 (36%)
pSN0	0	32 (64%)	32 (34%)
total	9 (18%)	37 (74%)	50 (100%)

table 1

Fifteen patients (30%) were upstaged, including nine cases from pN0 to pSN1 (4) or pSN2b (5) and six cases from pN1 to pSN2. Two of the pN0-pSN1 upstaged patients died with relapsed neck disease.

**Conclusions:** The SN biopsy technique with serial sections with immunohistochemistry appeared to be the best staging method in cN0 patients and provided evidence that routinely undiagnosed lymph node invasion may have clinical significance.

### 1178 Retrospective Review of Tonsil Specimens: Experiential Assessment of a Clinical Practice.

EL Courville, M Lew, PM Sadow. Massachusetts General Hospital, Boston; Massachusetts Eye and Ear Infirmary, Boston.

**Background:** Tonsil excision is a common surgical procedure performed in adults, primarily for recurrent infections and sleep apnea. Prior publications describe a low rate of malignancy in tonsil specimens, and the possibility of true occult malignancy is remote. At our institution, all tonsillectomy specimens from pts  $\geq 18$  yo are examined microscopically, while those from pts  $< 18$  yo are examined as gross only, and microscopy performed only with an abnormal gross finding or clinical suspicion for malignancy. The objective of our study was to provide institutional data regarding the prevalence of malignancy in adult bilateral tonsillectomy specimens and to promote discussion of best practices in pathology for tonsillectomy specimens based upon indication for surgery.

**Design:** In this retrospective study approved by our internal review board (2009P002643), we used our electronic medical record to obtain pathological diagnoses and clinical information for all adult tonsillectomy specimens processed in our pathology department over 45 consecutive months.

**Results:** Our department accessioned 1095 tonsil cases from adults, 80% of which consisted of bilateral excision specimens. Clinical indications for surgery included tonsillitis (72%), sleep apnea (12.5%), no clinical history available (11.5%), suspicion for malignancy (2.3%), asymmetry (1.7%), and mass (0.3%). Classification of the final pathology diagnoses for the 1746 specimens from bilateral excisions in adults is listed in Table 1. Of those specimens examined by histology, 1734 (99.8%) had a benign histopathologic diagnosis. The malignancy rate in bilateral adult tonsillectomy specimens at our institution over 45 months was 0.2% (two cases of squamous cell carcinoma and one case of follicular lymphoma). None of the malignant diagnoses were unexpected; therefore, our occult malignancy rate was 0%.

Table 1: Final Pathologic Diagnoses in Adult Bilateral Tonsillectomy Specimens

Benign	1682
No evidence of malignancy/Benign tonsillar tissue	52
Gross Diagnosis Only	8
Squamous Cell Carcinoma	2
Lymphoproliferative Disorder	2

**Conclusions:** Our data is consistent with data from a number of studies regarding the malignancy and occult malignancy rate in tonsil specimens. Based on this data, a gross diagnosis for adult bilateral tonsillectomy specimens removed for a clinical indication of tonsillitis or sleep apnea appears appropriate. However, consensus-derived practice guidelines regarding the triaging of adult tonsillectomy specimens will be needed prior to a change of practice.

### 1179 Significance of Ki-67 and p53 Immunopositivity in the Differential Diagnosis of Oral Necrotizing Sialometaplasia and Squamous Cell Carcinoma.

T Dadfarina, B Mohammad, MA Eltorky. UTMB, Galveston, TX.

**Background:** Necrotizing sialometaplasia (NS) is a benign inflammatory condition that usually involves the salivary glands in hard palate. It may be mistaken for a malignant neoplasm, such as invasive squamous cell carcinoma (SCC) as well as mucoepidermoid carcinoma. Despite the presence of some characteristic features that help distinguish NS from SCC on the H&E slides, some cases of NS with extensive pseudoepitheliomatous hyperplasia and reactive atypia of squamous epithelium with

necrosis are often difficult to differentiate from invasive SCC; hence, the application of immunohistochemistry has been attempted and proposed as an adjunct to diagnosis. In this study, we have demonstrated that p53, and Ki-67 staining may assist in the diagnosis of NS from SCC.

**Design:** Thirteen cases of NS, sixteen cases of well-differentiated and four moderately-differentiated invasive SCC related H&E slides in the head and neck with their matched tissue blocks were randomly selected from our surgical pathology archive from 1992 to 2009. Each case was stained with Ki67, p53, BCL-2, p16 and EGFR antibodies. The cases were reviewed by two pathologists independently.

**Results:** All thirteen cases of NS were negatively stained for BCL-2 and EGFR. Ki-67 staining was negative in all cases with only scattered nuclear staining in  $< 10\%$  of cells. Three cases (23%) showed weak and focal positive nuclear staining for p53. Two cases (15%) showed positive staining for p16. In sixteen well-differentiated SCC cases, p53 was considered focally & weakly positive in one patient (6%) and strongly positive in eleven patients (69%). BCL-2, p16, EGFR were positive in three cases (18%) and Ki-67 was positive with high nuclear staining  $> 35\%$  in all cases. In four moderately-differentiated SCC cases, p53 expression was positive in all cases. Two tumors (50%) had a positive expression of BCL-2. Three cases (75%) had a positive p16 staining and one (25%) had a positive EGFR staining. All cases were positive with high nuclear staining  $> 35\%$  of cells for Ki-67. The Ki-67 and p53 is generally more intense and is increased in higher grade of malignancy. The BCL-2, EGFR and p16 had the same pattern of staining with the same extent in NS and SCCs.

**Conclusions:** The diagnosis of NS may be difficult and is reliant upon a properly oriented section and a clinical history. Diagnosis may be supplemented via immunohistochemistry, demonstrating focal or absent immunoreactivity for p53, low to absent immunoreactivity for Ki-67 ( $< 10\%$  of cells). These findings may be helpful in confirming the diagnosis of NS in the appropriate clinical setting.

### 1180 Pitfalls in the Interpretation of p16 Immunohistochemistry and High-Risk HPV In Situ Hybridization in Head and Neck Cancer and Dysplasia.

P Devilliers, A Andea. University of Alabama, Birmingham.

**Background:** Immunohistochemical p16 expression (p16 IHC) may be used as a surrogate marker in the detection of high-risk human papillomavirus-associated head and neck cancer. High-risk HPV in situ hybridization (HR-HPV ISH) may be used as a confirmatory test on those cases positive for p16 IHC.

**Design:** A total of 130 cases of oropharyngeal cancer and dysplasia which had undergone HPV testing, as part of clinical care, during a 24 month period were selected, analyzed and correlated. HPV testing consisted of initial p16 IHC (MTM Laboratories, Westborough, MA), followed by high-risk HPV ISH (Ventana, USA).

**Results:** HPV status analysis was conducted on 100 oropharyngeal carcinomas and 30 cases of dysplasia. A total of 95% (95/100) of the tumors were positive for both p16 IHC and HR-HPV ISH, with the remaining 5% (5/100) positive for p16 and negative for HR-HPV ISH. Of the 30 cases of dysplasia, one third (10/30) consisted of mild dysplasia, which tested focally positive for p16 and negative for HR-HPV ISH. The remainder 2/3 (20/30) cases consisted of moderate to severe dysplasia, of which 27% (8/30) were focally positive for p16 and negative for HR-HPV ISH and 40% (12/30) had strong positivity for p16 and HR-HPV ISH. The staining pattern of p16 included cytoplasmic and nuclear. However, only those cases which were strongly positive for p16 nuclear and cytoplasmic staining were also positive for HR-HPV ISH. Regarding the staining pattern of ISH, areas of background staining of tissue not associated with the cells of interest were evident in all cases.

**Conclusions:** While incorporating p16 IHC and HR-HPV ISH to detect HPV in oropharyngeal carcinomas and severe dysplasias is a practical strategy, care ought to be taken when relying on the interpretation of p16 staining, considering positivity in cases showing more than 70% nuclear and cytoplasmic staining of the cells of interest. Dysplasias testing focally positive for p16, may be HR-HPV ISH negative. Moreover, cases of strong p16 IHC positivity which are negative for HR-HPV ISH may require further testing for wide spectrum HPV ISH.

### 1181 Utility of MAML2 Rearrangement Detection Using Fluorescent In-Situ Hybridization To Distinguish Glandular Odontogenic Cyst from Central Mucoepidermoid Carcinoma.

JJ Garcia, EL Barnes, K Cieply, S Dacic, RC Jordan, RR Seethala. University of Pittsburgh Medical Center, PA; University of California, San Francisco.

**Background:** Glandular odontogenic cyst (GOC) poses diagnostic challenge because of its histomorphologic overlap with central mucoepidermoid carcinoma (CMEC). While the diagnostic value of the t(11;19) *MECT1-MAML2* fusion gene has been established in distinguishing MEC from other neoplasms of the head and neck (Warthin's tumor, oncocytoma, adenosquamous carcinoma, salivary duct carcinoma), its potential use to discriminate CMEC from GOC has not been delineated.

**Design:** Nine cases of GOC (defined as multilocular cystic lesions lined by non-keratinized epithelium with focal plaque-like thickening and an occasional whorling appearance, scattered mucous cells and duct-like structures) and two cases of CMEC were examined. Available clinical and pathologic parameters were obtained. Cases were evaluated for *MAML2* rearrangements by fluorescent in-situ hybridization (FISH) using a *MAML2* – 11q21 break apart probe (SpectrumGreen-labeled BAC probe RP11-676L3 and SpectrumOrange-labeled BAC probe RP11-16K5; Children's Hospital Oakland Research Institute, Oakland, CA, USA) spanning the entire chromosome region of the *MAML2* gene. At least 60 interphase tumor cell nuclei were evaluated per case. A minimum of 20% of cells with split signal was considered positive.

**Results:** All nine cases of GOC were gnathic (6 mandible, 2 maxilla, 1 not specified) with a median age of 56.7 years (range: 43-87) and a male to female ratio of 8:1. None

of the 9 cases (0%) showed a *MAML2* rearrangement by FISH. Cases of CMEC occurred in the mandibles of a 61-year-old male and 40-year-old female. Both cases of CMEC were low-grade and showed *MAML2* rearrangement (100%).

**Conclusions:** In contrast to CMEC, *MAML2* rearrangements were not detected in GOC; therefore, FISH serves as a potentially useful diagnostic tool in discriminating GOC from CMEC.

### 1182 Histopathology and TCR Clonality in 5 Cases of Eosinophilic Ulceration of the Oral Mucosa.

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**Background:** The Eosinophilic Ulcers of the Oral Mucosa (EUOM) are benign lesions that affect the oral cavity, predominantly the tongue. However, histopathological and phenotypical features are worrisome and may lead to a misdiagnosis of malignancy. The aim of our study was to characterize the clinical, pathological and clonality features of five new cases of EUOM.

**Design:** 5 cases that fulfilled the criteria for the diagnosis of EUOM were retrieved from CNIO consultation files. A detailed immunohistochemical study together with clonality testing for TCR rearrangements with Biomed2 primers was done.

**Results:** A summary of the results is shown in Tables 1 & 2. All patients were male with age between 23 to 74 years-old. The location of the lesions was the lateral margin of the tongue. Pathological evaluation showed in all cases a prominent inflammatory polymorphic cell population with invasive features and predominantly composed of lymphocytes, histiocytes and eosinophils. Mild necrosis was seen and the mitotic rate was generally low. By immunohistochemistry it is shown a predominance of T-cells (CD3+, CD4+, CD30+, TCR betaF1+), with scattered B cells (CD20+). The PCR analysis for TCR rearrangements was polyclonal for all loci tested in all but one case that showed a single monoclonal peak in TCR G1.

TABLE 1

Case Number	Age y/o	Sex	Location	Deep involvement	Cellular atypia	Atypical Mitosis
1	46	Male	Tongue lateral margin	Soft tissue / Muscle	YES	8/10 HPF
2	23	Male	Tongue	Soft tissue / Muscle	NO	1/10 HPF
3	31	Male	Tongue lateral margin	Soft tissue / Muscle	NO	3/10 HPF
4	50	Male	Tongue lateral margin	Soft tissue / Muscle	NO	1/10 HPF
5	74	Male	Tongue lateral margin	Soft tissue / Muscle	NO	1/10 HPF

Clinical and pathological features

TABLE 2

Case Number	CD3	CD30	TCR betaF1	CD4	CD8	Clonality
1	+++	++	+++	+++	++	TCR G1 clonal. G2 Polyclonal
2	++	-	+++	++	++	NV
3	+++	++	+++	++	+	TCR non clonal (G1, G2, B1, B2)
4	++	+	+++	+++	-	TCR Polyclonal (G1, B1, B2)
5	+	+	+++	+++	-	TCR Polyclonal(G2, B2)

Immunohistochemical and clonality features

**Conclusions:** The EUOM may resemble morphologically a malignant lesion, particularly T-cell lymphoma. A detailed phenotypic and clonality study helps to confirm the reactive nature of the infiltrate in most instances. However few cases may show monoclonal T cell populations that do not necessarily indicate malignancy in this particular context.

### 1183 Angiomatoid Nasal Polyp. Often Misdiagnosed and Little Known Lesion. Report of 45 Cases.

L Hadravsky, A Skalova, M Michal. Charles University in Prague, Czech Republic.

**Background:** Angiomatoid nasal polyps (ANP) arising from inflammatory nasal polyps are benign lesions with frequent recurrences. Inflammatory nasal polyps may become partially or extensively infarcted which results in hemorrhage, necrosis and erosion of the surrounding tissues including the skeletal bones. These changes in ANP may cause the histological resemblance to various benign and malignant tumors of nasal and perinasal regions and cause misdiagnosis which may lead to destructive therapeutic approach.

**Design:** 45 cases of Pilsner consultation registry (32 men and 13 women) were accessed to the registry between 1994-2010 were retrieved. The follow-up was available in 27 patients.

**Results:** The average age of patients when ANP detected was 49 (13-84) years in men and 54.3 (18-83) years in women. The most common reported symptoms were: recurrent epistaxis (21/27), obstruction (16/27), secretion of mucus and pus (5/27), and pain located to the paranasal sinuses (3/27). The average size of ANPs was 25,71 (5-80) mm. Most frequent locations were: nasal septum (14/41), antrum Highmori (12/41), ethmoid sinuses (5/41) lateral wall of nasal cavity (5/41), sphenoid sinus (1/41), and non-specific nasal cavity (4/41). X-ray or computed tomography scans were performed in 19 cases and bone erosions/deviations occurred in 4/4 cases of them. Allergies were found in 44% (11/25) patients. Most common allergies reported were: dust, mites, pollen, hey, honey, ATBs, nuts, chlorine, and cold. Six patients had a history of cancer (endometrial adenocarcinoma, rectal carcinoma, colonic carcinoma, carcinoma of kidney, lymphoma, and plasmacytoma and cutaneous basal cell carcinoma). Initial misdiagnoses submitted by referring pathologists were reported in 20/45 cases (angiofibroma 32%, hemangioma 24%, hemangiopericytoma 16%, angiosarcoma 12%, pyogenic granuloma and hemangioidendelioma- both at 8%). None of the patients died of the disease and there has been no progression in any patient. Recurrence was recorded in 30% (9/30).

**Conclusions:** This study confirms that ANPs are benign, often recurring lesions, likely to be confused with other tumorous lesions of sinonasal region by pathologists, which

may lead to useless destructive therapeutic approach. There are no clinicopathologic studies describing these lesions available in the literature, which contributes to the rate of misdiagnoses.

### 1184 Transcriptionally Active High-Risk Human Papillomavirus in Salivary Mucoepidermoid Carcinoma – A Novel Finding.

T Isayeva, S Bai, N Said-Al-Naief, D Gnepp, M Brandwein-Gensler. UAB, Birmingham; University of the Pacific, San Francisco, CA; Rhode Island Hospital, Providence.

**Background:** Mucoepidermoid carcinoma (MEC) is a common salivary tumor of unknown pathogenesis. Epidemiological and molecular data have established high-risk human papillomavirus (HR HPV) in the pathogenesis of many oropharyngeal squamous carcinomas. Interestingly, a recent analysis of the SEER data suggests that the incidences of oral/oropharyngeal MEC are significantly increased in young females from 1975 to 2005. Here we test the hypotheses that HPV is involved in pathogenesis of MEC, with increasing contribution over time.

**Design:** This exploratory study included consecutive patients from 1980-2010 with confirmed diagnoses, and available paraffin-embedded specimens. Morphologically-guided 1 mm tumor cores were harvested from the paraffin blocks and deparaffinized, DNA was extracted with phenol-chloroform and precipitated with ammonium acetate; total RNA was isolated with TRIzol and precipitated with isopropyl alcohol. DNA and RNA were treated with RNase and DNase respectively. RNA was reverse-transcribed using cDNA synthesis kit. **HPV16 / HPV18 E6 / E7** transcripts were measured by **PCR** and **RT-PCR**. Quantitative PCR was performed using Bio-Rad SYBR green supermix and Opticon2 detection system. Assays were performed in triplicate with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and beta-actin internal controls to confirm specimen suitability. HeLa and SiHa cells were the positive controls for HPV18 and HPV16, respectively. All specimens negative for HR HPV and GAPDH/B-actin transcripts were deemed unsuitable and excluded.

**Results:** We had suitable specimens from 90 patients, ages 17-85, (mean 49); 70 tumors were intraoral, 16 were parotid-based, and 2 arose in the submandibular and sublingual glands, respectively. At least one HR HPV transcript was detected in 56 of 90 tumors; 8 MEC had both HPV16 E6/E7 transcripts, 6 had both HPV18 E6/E7 transcripts, and 2 MEC had all four transcripts (HPV16/HPV18 E6/E7). MEC diagnosed in 2000 or later were significantly more likely to harbor at least one HR HPV transcript as compared to MEC diagnosed prior to 2000 (p = 0.0467, Fischer two-tailed exact test).

**Conclusions:** These intriguing results demonstrate that transcriptionally active HPV16/18 can be detected in MEC, and are more common in tumors diagnosed after 2000. Thus it is likely that HR HPV may be an increasing contributor to the pathogenesis of MEC; it is unlikely that RNA degradation in older specimens spuriously contributed to this finding, as all specimens had confirmed housekeeping internal controls.

### 1185 p16 Positive Squamous Cell Carcinomas of the Larynx Are Predominantly Located in the Supraglottis Compared to Glottic and Subglottic Locations.

SM Kirby, M Leon, H Iwenofu. The Ohio State University, Columbus.

**Background:** The significance of human papillomavirus (HPV) in squamous cell carcinomas (SCC) of the head and neck is well established, particularly in oropharyngeal cancers. Expression of p16 is strongly associated with HPV infection and its utility as a surrogate marker has been validated in previous studies. There is precedent literature on HPV associated laryngeal carcinomas but its anatomic distribution within the larynx has not been previously studied. Herein, using p16 immunohistochemistry, we examined expression in laryngeal SCC relative to supraglottic, glottic, and subglottic locations.

**Design:** A computerized search yielded 150 patients with squamous cell carcinoma of the larynx at the Ohio State University Medical Center. Cases which could not be identified as solely supraglottic, glottic, or subglottic were eliminated from the study. The remaining cases (n=101) were sorted into supraglottic (n=46), glottic (n=52), and subglottic (n=3) groups. Representative sections of tumor were used to construct a tissue microarray (TMA), including two 1.5 mm cores of tissue per case. The TMAs were stained for p16 using routine methods in a DAKO automated stainer. The results were then evaluated semiquantitatively for intensity (0-3+) and percent of tumor cells stained. Staining of <5% was considered negative.

**Results:** Expression of p16 was found in 18% of all laryngeal SCCs. Supraglottic tumors showed a higher rate of 30% (14/46) with 8 cases staining >75% of the tumor cells with 2-3+ intensity. Glottic tumors had a rate of 8% (4/52) with more focal distribution. 1 of 3 (33%) subglottic tumors was positive with staining of <10% of cells. The results are summarized below. The difference in p16 expression for supraglottic and glottic groups were statistically different (p<0.05).

	Positive	Negative
Supraglottic (n=46)	14	32
Glottic (n=52)	4	48
Subglottic (n=3)	1	2

**Conclusions:** Our study clearly indicates a higher proportion of p16 positive SCC in the supraglottic larynx (30%) compared to 18% in all laryngeal locations. As p16 is a surrogate marker for HPV association; this finding may be explained by a higher rate of HPV involvement in SCC of supraglottic origin. This might be related to its proximity to the oropharyngeal site. Further studies are needed to define this.

### 1186 Molecular Characterization of Head and Neck Basaloid Squamous Cell Carcinoma (BSCC).

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**Background:** BSCC was originally described as a rare aggressive subtype of head & neck squamous cell carcinoma (HNSCC). The diagnosis remains controversial due to overlapping histologic findings with poorly differentiated non-keratinizing

SCC (NKSCC) and lack of diagnostic reproducibility. We explore distinct molecular pathways which may aid in the diagnostic stratification and prognostic relevance of HNSCC with basaloid features.

**Design:** The HNSCCs originally diagnosed as BSCC or SCC with basaloid features from 1990-2010 were analyzed. These were reviewed and reclassified using strict criteria: BSCC: solid, lobular, nested growth pattern, small cells with scant cytoplasm, hyperchromatic nuclei with peripheral palisading, foci of central coagulative necrosis, stromal hyalinosis, abrupt keratinization. Otherwise, cases were classified as NKSCC. Tissue microarray were constructed and the following immunohistochemical stains were done: MIB-1, p53, p16, Cyclin D1, BCL-2, EGFR. High risk HPV was detected by chromogenic in-situ hybridization (CISH) using cocktail probe (genotypes-16, 18, 33, 35, 45, 51, 52, 56, 66). Contingency table analysis of correlation between HPV status and expression of proteins was performed using Fisher's exact test.

**Results:** Total of 30 cases were retrieved (14-BSCC, 16-NKSCC). Results of the CISH & IHC and correlation between p16 and HR-HPV are summarized in table 1 and 2, respectively. There was high MIB-1 proliferation index in all the cases. Bcl-2 protein was upregulated in 97% of cases.

Table 1

	HPV-pos	P16-pos	P53-pos	CYCLIND1-pos	EGFR-pos
BSCC	9/14, 64.3%	7/14, 50%	9/14, 64.3%	8/14, 57.1%	11/14, 78.6%
NKSCC	5/15, 33.3%	10/16, 62.5%	14/16, 87.5%	4/16, 25%	7/16, 43.8%
P-value	0.0954	0.4884	0.1336	0.0732	0.0522

Table 2

	HPV-neg	HPV-pos	Total
p16neg	6	6	12
p16pos	9	8	17
Total	15	14	29

**Conclusions:** BSCC of HNSCC are frequently positive for HR-HPV and tumors arising in Waldeyer's ring are 100% positive, indicating HPV driven carcinogenesis. Most of these cases did not have p53 dysregulation. In addition, EGFR was significantly upregulated in BSCC. p16 as surrogate marker for HPV underestimates HPV+ status in Waldeyer's ring BSCC. In contrast, NKSCC appear to have different molecular pathway where p16 is frequently positive but with only 47% HPV+ and negative correlation with EGFR expression. Moreover, p53 is positive in over 87% of NKSCC. We conclude that the molecular pathway of HNSCC differs according to site and morphology. Positive predictive value of p16 regarding HPV status appears to be site and morphology specific.

**1187 Role of High Risk Human Papilloma Virus (HR-HPV) in Carcinogenesis of Spindle-Cell (Sarcomatoid) Squamous Cell Carcinoma of the Head and Neck.**

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**Background:** Spindle-cell (sarcomatoid) squamous cell carcinoma (sSCC) of head and neck, a variant of conventional squamous cell carcinoma (cSCC) is rare but aggressive neoplasm. There have been many published reports exploring different molecular pathways in cSCC but data is limited for sSCC. In this study we explored possible role of high risk human papilloma virus infection (HR-HPV) and its association with p16, p53, Cyclin D1, MIB-1 proliferation index and EGFR expression.

**Design:** We identified 14 cases of sSCC from 2001-2008. The light microscopic features were reviewed and diagnosis of sSCC was rendered to tumors either entirely composed of pleomorphic spindle cells or having a distinct spindle cell component in cSCC. Tissue microarray block was constructed and following immunostains were done: p16, p53, Cyclin D1, EGFR, MIB-1. High risk HPV was detected by chromogenic in-situ hybridization (CISH) using cocktail probe (genotypes-16, 18, 33, 35, 45, 51, 52, 56, 66). Contingency table analysis of correlation between HPV status and expression of proteins was performed using Fisher's exact test. Clinical data was recorded.

**Results:** Nine patients (64.2%) presented with clinical stage IV, 3 (28.6%) stage I at presentation. For 2 patients clinical stage was unknown. All stage I tumors were in vocal cords, whereas stage IV disease involved larynx and Waldeyer's ring. None of the patients with stage I had disease progression after a follow-up of more than 3 years. Follow-up was available for 8 patients with stage IV cases; 5 of them succumbed to carcinoma, 2 died because of unrelated cause, and one is alive following a 2 year postoperative period. Five cases (35.7%) were p16+, 12 cases (85.7%) were HR-HPV+, p53 was mutated in 11 cases (78.6%), Cyclin D1 was upregulated in 2 cases (14.3%), and only 1 case (8%) had overexpression of EGFR. MIB-1 labeling was high in all the cases.

**Conclusions:** In our cohort there was an overwhelming number of sSCC which were HR-HPV+ with overexpression of p53, p16 and negative EGFR and Cyclin D1 expression. Our results indicate that aggressive behavior of sSCC is in part related to association of two carcinogenesis pathways viz. HR-HPV+ and p53 mutation unlike HR-HPV+/p53- cSCC which has more favorable clinical behavior. Interestingly, we did not find association between HR-HPV+ status and overexpression of EGFR which we found in cSCC in a separate cohort. p16 has limited value as a surrogate marker for HR-HPV status in sSCC because of low sensitivity (41.7%).

**1188 Histologic Features of Oral Squamous Cell Carcinoma Invasive Front Correlate with Genetic Markers of Survival and/or Metastasis.**

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**Background:** Histologic features of oral squamous cell carcinoma (OSCC) previously associated with biologic outcome include nuclear pleomorphism, keratinization, cell

organization, and inflammation at the invasive front. We (Oralchip study, NIH/NCI 095419) previously used fresh tumor tissue and Affymetrix genechips to identify gene expression markers associated with survival and/or metastasis. Expression levels of these markers have not been previously correlated with histologic features.

**Design:** Two pathologists blinded to the OralChip results independently examined H&E slides from 84 OSCC patients for 21 histologic features. Discrepant results were re-examined jointly. Histologic results were correlated with marker expression values from corresponding fresh tumor samples.

**Results:**

Markers of:	Histologic feature (p-value ≤)
<b>Decreased Survival</b>	
<i>LAMC2</i>	Prominent desmoplasia (0.002)
	Eosinophils present (0.021)
	Myxoid stroma (0.039)
	Increased nuclear pleomorphism (0.044)
<i>OSMR</i>	Myxoid stroma (0.005)
<i>SERPINE1</i>	Dissociated/non-cohesive growth (<0.001)
	Myxoid stroma (0.001)
	Perineural invasion (0.001)
	Basaloid features absent (0.016)
	Prominent desmoplasia (0.037)
<i>OASL</i>	Absence of basaloid features (0.005)
	Increased keratinization (0.024)
<i>SLC16A1</i>	Increased keratinization (0.002)
	Prominent squamous eddies (0.004)
	Myxoid stroma (0.008)
	Eosinophils present (0.014)
	Granulomatous response absent (0.031)
	Prominent desmoplasia (0.042)
<b>Metastasis</b>	
<i>MYO5A</i>	Increased keratinization (<0.001)
	Basaloid features absent(0.001)
	Prominent squamous eddies (0.004)
<i>RNF145</i>	Decreased keratinization (0.001)
	Histiocyte response absent (0.010)
	Basaloid features present (0.037)
<i>FBOX32</i>	Perineural invasion (0.008)
	Eosinophils absent (0.047)
<i>CTONG2002744</i>	Prominent squamous eddies (0.022)

**Conclusions:** We identified histologic features at the OSCC invasive front that are significantly associated with genetic markers of decreased survival and/or risk of metastasis. Further investigation to explore these associations is warranted.

**1189 Recognition of Nonkeratinizing Morphology in Oropharyngeal Squamous Cell Carcinoma – A Prospective Cohort and Interobserver Variability Study.**

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**Background:** Nonkeratinizing morphology in oropharyngeal squamous cell carcinoma (NK SCC) is increasingly recognized as strongly correlating with p16 expression. However, NK SCC is not widely recognized by pathologists. We have developed a histologic classification system typing tumors as type 1 (keratinizing SCC), type 2 (hybrid SCC), or type 3 (NK SCC). Our aims were to 1) Correlate the histologic types with p16 expression and 2) Examine the reproducibility of diagnoses for a group of head and neck pathologists applying these criteria.

**Design:** For aim 1, we captured 6 months of prospective data on routine, clinical SCC cases which were classified using the histologic criteria by the 3 individual central institutional pathologists. For aim 2, from a large research database, 40 test cases were randomly selected and an instruction sheet and 3 example slides circulated for review to 6 head and neck pathologists not previously familiar with the system. p16 immunohistochemistry was performed on all cases with 50% of tumor cells (nuclear + cytoplasmic) staining as the lower cutoff for positivity.

**Results:** For aim 1, there were 51 cases. All cases called type 3 were p16 positive.

Prospective Cohort: Histology-p16 Correlation

Type	Total Cases	p16 positivity
Keratinizing (1)	13	5 (38.5%)
Hybrid (2)	12	12 (100%)
Nonkeratinizing (3)	26	26 (100%)

For aim 2, agreement was highest between the 6 pathologists for types 1 and 3 (kappa values 0.62 and 0.56, respectively, considered moderate to good agreement; p<0.0001 for both) and lowest for type 2 (kappa 0.35, considered poor agreement; p<0.0001). 33 of the 40 cases were p16 positive. Every case classified as type 3 by any reviewer (21 cases) was p16 positive.

**Conclusions:** Head and neck pathologists can recognize NK SCC as a distinct subtype of oropharyngeal SCC. In particular, our data again show that when a pathologist classifies a tumor as type 3, this reliably predicts p16 positivity.

### 1190 Middle Ear Adenomas Contain Focal Areas with Basal Cells but Not Myoepithelial Cells.

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**Background:** Middle ear adenomas (MEAs) are rare neoplasms generally considered to be benign. The true nature of these neoplasms is unknown but are considered to be along a spectrum with carcinoid tumors. To date, immunohistochemical staining for myoepithelial markers has not been reported in these lesions.

**Design:** The archives of one institution were retrospectively searched for tumors arising within the middle ear which had material available for immunohistochemical staining. Six cases of MEA and four cases of jugulotympanic paraganglioma (JTG) were identified. An additional two cases of ceruminous adenoma were included for comparison. Immunohistochemical staining was performed for the following markers: smooth muscle actin, p63, S100 protein, cytokeratin 5/6, and cytokeratin 7. The pattern of immunohistochemical staining within each tumor was recorded as negative (<1% of cells), focal (<25% of cells) or diffuse (>25% of cells), and sub-classified as luminal or abluminal where appropriate.

**Results:** The MEAs showed a range of morphologic patterns including cribriform, trabecular, nested and/or glandular architecture. Crush artifact was common in all biopsies. Immunohistochemical staining was focally to diffusely positive for CK7 in all cases, with accentuation in areas with glandular architecture. CK 7 was positive in a predominantly luminal pattern in the glandular areas. The abluminal cells were focally positive for CK5/6 and p63 in the glandular areas. S100 was focally positive in one case, and the MEAs were uniformly negative for SMA. JTGs displayed lobulated nests of cells centered on vascular structures. Immunohistochemical staining was diffusely positive for S100 with accentuation of sustentacular cells, and CK7, CK5/6, SMA and p63 were uniformly negative. Ceruminous adenomas showed gland formation with eosinophilic luminal secretions. Immunohistochemical staining for CK7 was diffusely positive in a luminal pattern, and CK5/6, p63, S100, and SMA were diffusely positive in the abluminal cells.

**Conclusions:** The immunohistochemical profile of MEAs suggests that these tumors harbor at least 2 cell populations, including luminal and basal cells, albeit focal. However, unlike ceruminous adenomas, MEAs lack true myoepithelial differentiation given the absence of S100 and SMA staining in all cases.

### 1191 Small Biopsy Specimens Reliably Indicate p16 Expression Status of Oropharyngeal Squamous Cell Carcinoma – A Biopsy and Resection Specimen Correlation Study.

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**Background:** Human papillomavirus (HPV)-related squamous cell carcinoma (SCC) is a morphologically and clinically distinct type of oropharyngeal SCC. Tumor HPV status on initial diagnostic biopsy is critical for proper management, and p16 immunohistochemistry has emerged as a reliable surrogate marker for HPV status. We sought to study if p16 staining of small diagnostic biopsies reliably predicts that of whole tumor resection specimens.

**Design:** From a database of oropharyngeal SCC for which p16 immunohistochemistry and histologic typing was already performed, all cases which underwent surgical resection and for which an in house primary tumor biopsy specimen was available were selected. Histologic typing was as follows: Type 1, keratinizing SCC, Type 2, hybrid SCC, and Type 3, nonkeratinizing SCC. p16 immunohistochemistry was then also performed on the diagnostic biopsy specimens. Staining was graded in the same manner on both biopsies and resections. In particular, staining was nuclear and cytoplasmic and was graded as follows: 0 = negative; 1+ = 1 to 25% of cells positive; 2+ = 26 to 50%; 3+ = 51 to 75%; 4+ = 76 to 100%.

**Results:** Biopsy staining results were as follows:

p16 Staining Results by Histologic Type					
p16 IHC	0 (%)	1+ (%)	2+ (%)	3+ (%)	4+ (%)
All biopsies (32)	8 (25.0%)	1 (3.1%)	1 (3.1%)	1 (3.1%)	21 (65.6%)
Type 1 (9)	7 (77.8%)	1 (11.1%)	1 (11.1%)	0 (0%)	0 (0%)
Type 2 (7)	1 (14.3%)	0 (0%)	0 (0%)	1 (14.3%)	5 (71.4%)
Type 3 (16)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	16 (100%)

Strictly considering the p16 score, biopsy-resection correlation was present in 28 (85.0%) of all cases including 6 (66.6%) of Type 1, 6 (85.7%) of Type 2, and 16 (100%) of Type 3 cases. Among Type 1, there were two cases that differed as p16 0 versus 1+ and one case that differed as p16 1+ versus 2+. For Type 2, the one discrepant case differed as p16 3+ versus 4+. Considering p16 expression binarily as 50% or more (3+ or 4+) being positive and lesser amounts (0, 1+, or 2+) being negative, there was perfect biopsy-resection correlation for all 32 cases. With p16 expression in resection specimens considered the gold standard, positive p16 staining in biopsies was both 100% sensitive and specific.

**Conclusions:** Our results demonstrate that p16 staining in diagnostic biopsies reliably reflects whole tumor staining results. p16 immunohistochemistry can be used on small diagnostic biopsies to assess for tumor HPV status.

### 1192 Molecular Analysis of HPV 16/18 in Patients with Oropharyngeal Squamous Cell Carcinoma from an Inner City Hospital.

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**Background:** Human papillomavirus (HPV) plays a role in the development of a subset of head and neck cancers such as squamous cell carcinoma (SCC) of oropharynx, particularly tonsillar and base of the tongue with basaloid and non-keratinizing morphology. Molecular evidence shows that 25-30% of tumors are positive for high-risk HPV subtypes, with HPV-16 detected in 95% of cases. Studies have shown that incidence of HPV-associated oral cancers varies with ethnicity. Reports suggest that patients with HPV-positive oral cancers have better prognosis than HPV-negative

tumors. We analyzed tumors from patients with oropharyngeal SCC for the presence of HPV subtypes. In addition we studied the status of p16, an important cell cycle regulator in tumors.

**Design:** Archived formalin fixed paraffin embedded tissue specimens from 100 patients (72 male and 28 female) with oropharyngeal SCC were analyzed. The average age of the patients was 59 years. The study included 41% Caucasian, 24% Hispanic, 20% African-American, and 2% Asian subjects. DNA was extracted from microdissected tumor enriched tissue and real-time amplification was performed on the ABI 7500 with probes specific for HPV 16/18. PCR products were sequenced for confirmation of HPV-16/18. Amplification of the beta-globin gene served as DNA control. Tissue sections were immunostained with a commercially available antibody to p16<sup>INK4a</sup>.

**Results:** Real-time PCR assays for the detection of HPV 16/18 were validated using positive cytology specimens. HPV-16 positive SiHa and HPV-18 positive HeLa cell lines. HPV 16 was detected in 27% of patients with oropharyngeal SCC and HPV-18 in 2% of specimens. One specimen was positive for both HPV 16/18. The rates of HPV positivity were comparable for males and females. 39% of the positive specimens belonged to Caucasians and 25% to Hispanics. In contrast HPV 16 was detected in only 5% of specimens from African-American subjects. Immunohistochemistry showed the presence of p16 in 68% of the cases. Most of the specimens (89%) infected with HPV stained positive with the antibody to p16.

**Conclusions:** Our results show that 27% of oropharyngeal SCC tumors were positive for HPV with HPV 16 being the foremost subtype. HPV positivity was not associated with gender. Caucasians were more likely to have HPV associated with SCC followed by Hispanics. African-American subjects were least likely to have HPV positive oropharyngeal tumors. The p16 expression localizes to HPV-positive cancers. These observations may have important implications for the etiology and follow-up of patients with oropharyngeal cancer.

### 1193 PET-CT Imaging in Lesions of the Tonsil for the Detection and Staging of Squamous Cell Carcinoma: A 3 Year Experience in 43 Cases.

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**Background:** Positron Emission Tomography-Computerized Tomography (PET-CT) has a role in the staging of head and neck (H&N) squamous cell carcinoma (SCCs). In the pharyngeal palatine tonsil (PPT), it has been reported that there may be up to a 40% false positive rate. In the PPT, the calculated standardized relative uptake values (SUVs) on PET-CT is 3.48 +/- 1.3 in normal patients. It is reported that an SUV difference between right and left of 0.83 or > will pick up 100% of occult SCCs.

**Design:** We searched our files for tonsil biopsies performed since 2005. We reviewed the clinical, radiographic, pathologic findings. PET-CT was performed in selected patients using 18F-FDG PET fused with a low dose non-contrast CT scans. The tonsil location SUVs were recorded and compared to pathologic data. Other sites of increased SUV uptake were also recorded.

**Results:** There were 901 patients with tonsil histology. There were 43 patients with PET-CT scans. The SUV values in the PTTs ranged from 0 to 35. There were 19 biopsy negative cases which showed either reactive hyperplasia or atrophy (Table 1). All cases with SCC in the H&N region were diagnosed prior to the PET-CT scan via tonsil biopsy or biopsy of another site (lymph node or another H&N location). No occult SCCs were found with PET-CT. There were 2 cases (SUV 0) in which the biopsy showed SCC. SUV values between the right and left tonsils did not predict cancer. The patients with SCC SUVs ranged from 0-35 and patients without SCC ranged from 2.8-8.2 (Table 1). Over 33% of patients with a high SUV on PET-CT had no SCC at tonsil biopsy. In tonsil SCC, there was a false negative rate of 35%.

Table 1: SUV to Tonsil Biopsy Diagnosis

SUV number	Tonsil with SCC	Tonsil Negative
Increased	14	12
Normal range	8	7
Equivocal	1	0

**Conclusions:** PET-CT is useful in the diagnosis, staging and prognosis of H&N SCC but is not as useful in PPT sites due to inherent lymphoid tissues that may be atrophic or reactive with varying SUVs. An extremely high SUV (>8) may be of value but SUVs of 3-8 may be fraught with false positive and false negative diagnoses. It should be known that occult squamous cell carcinoma may have no SUVs and biopsies in patients with metastatic SCC in the neck should have all locations in the H&N biopsied despite PET-CT findings of low values. It has been our experience that 100% of our patients without known biopsy proven SCC in the H&N regions had only reactive lymphoid tissue at PTT biopsies directed by PET-CT.

### 1194 HGMA2 Is Overexpressed in Epithelial Precursor Lesions and Oral Squamous Cell Carcinoma.

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**Background:** Oral squamous cell carcinoma (OSCC) is often associated with epithelial precursor lesions (EPL), most commonly oral squamous dysplasia (OSD). OSD grading is an important but imperfect predictor of the malignant potential of various EPL. Identification of molecular markers to risk-stratify EPL and/or detect early OSCC is clinically relevant. High mobility group protein HMGA2 mediates epithelial-mesenchymal transition and tumor invasiveness of thyroid, pancreas, stomach and lung carcinomas, as well as OSCC. HMGA2 expression in EPL has not been investigated to date. We aimed to assess HMGA2 protein expression in EPL and OSCC and compare it with that in benign epithelium.

**Design:** The study included 69 areas present in 51 excisional samples from 48 patients, evaluated by 2 pathologists who independently graded dysplasia using WHO classification. Consensus diagnoses were grouped for statistical analysis as follows:



normal mucosa, low-grade lesions (LG; mild dysplasia), high-grade lesions (HG; moderate dysplasia, severe dysplasia, carcinoma in-situ), and microinvasive carcinoma (MI). 61 additional cases of OSCC from a tissue microarray were also assessed. Only nuclear HMGA2 staining was recorded and scored independently by 2 observers by multiplying staining intensity (0-3) with the percentage of positive cells (1 for <5%, 2 for 5-50%, 3 for 51-80%, 4 for >80%). Using 8 samples of histologically uninvolved mucosa and 7 samples of oral mucosa excised from non-SCC tumors, a HMGA2 score of 6 or more was considered positive for this study. Statistical analysis was performed using chi-square test with  $p < 0.05$  for statistical significance.

**Results:** The 69 samples comprised areas of interest as follows: 15 normal mucosa, 11 LG, 35 HG and 8 MI. Nuclear HMGA2 expression was detected in only 1 of 15 (7%) normal mucosa samples, but in 6 of 11 (55%) LG, 21 of 35 (60%) HG, 3 of 8 (38%) MI and 47 of 61 (77%) OSCC samples. When compared with normal mucosa, HMGA2 was significantly overexpressed not only in OSCC ( $p < 0.001$ ), but also in LG ( $p = 0.007$ ) and HG ( $p < 0.001$ ) EPL. The borderline significance for MI ( $p = 0.06$ ) might be due to small sample size.

**Conclusions:** Our data shows that HMGA2 is overexpressed not only in OSCC, but also in LG and HG EPL and early invasive carcinoma. Overall, HMGA2 nuclear expression increased with the severity of dysplasia in EPL, and was highest in OSCC, suggesting that HMGA2 involvement in the neoplastic progression of EPL to OSCC might occur at an earlier stage than previously thought.

#### 1195 Does p16 Immunostaining Increase the Detection of Histologically Minute Squamous Cell Carcinoma of the Upper Aerodigestive Tract?

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**Background:** Head and neck squamous cell carcinoma (SCC) not infrequently presents with metastatic disease for which no clinically obvious primary malignancy is identified. In such cases, pathologists are often asked to evaluate speculative biopsies of the upper aerodigestive tract for minute primary tumors to help guide surgical and radiation therapy. It has been previously shown that a significant proportion of these primary tumors are from the oropharynx, many secondary to infection by human papillomavirus (HPV). It has also been shown that p16 immunohistochemistry is an excellent surrogate test for HPV-induced SCC. We speculated that p16 immunostaining might assist in the detection of very small HPV-induced SCCs of the oropharynx that might otherwise be overlooked with conventional staining.

**Design:** Fifty-one patients were identified with head and neck SCC who had undergone biopsies of the upper aerodigestive tract that had been originally interpreted as negative for SCC. All biopsies and palatine tonsil excisions were reviewed. Immunohistochemistry was performed with antibodies to p16 on all tissues. All positive-staining cases were reviewed a second time for small or low-grade lesions.

**Results:** There were a total of 156 samples from the 51 patients evaluated with p16 immunohistochemistry. Of those, 143 (92%) were negative for p16 (or showed positive staining of normal appearing reticular tonsillar crypt epithelium, as previously described). Ten (6%) showed focal p16 immunoreactivity that correlated with histological changes consistent with low-grade squamous intraepithelial lesions similar to those of the cervix. Three (2%) showed p16 immunoreactivity that correlated with histological changes consistent with SCC in situ. Eight of the low-grade lesions were in patients whose original biopsies were read as entirely negative, while the other two were found in patients who also had intraepithelial lesions seen in other biopsies. Of the cases with SCC in situ, all had been originally diagnosed as atypical or dysplastic.

**Conclusions:** Immunohistochemical staining with antibodies to p16 did not improve detection of invasive cancer in cases of head and neck SCC of unknown primary. It did reveal occasional, small low-grade intraepithelial lesions that were missed on initial examination. Given the submission of entire palatine tonsils in many cases, these results suggest that some of the small primary malignancies in these cases may have entirely regressed.

#### 1196 Papillary Thyroid Carcinomas with Warthin and Tall Cell Features: A Review of 21 Patients.

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**Background:** The Warthin-like variant of papillary thyroid carcinoma (WLV-PTC), is characterized by the presence of a papillary proliferation of oncocytic follicular epithelium showing nuclear cytology of PTC; the cells are arranged on fibrovascular cores containing a dense lymphoplasmacytic infiltrate. The prognosis of these lesions is excellent. We describe the clinicopathologic features characteristics of a cohort of patients in which WLV-PTC was intimately associated with tall cell variant (TCV) of PTC, a known aggressive variant.

**Design:** The computerized pathology case archives and the authors' consultation files (from 1985 -2010) were searched for cases with a diagnosis of WLV-PTC and PTC with WL areas. The available histologic slides were evaluated and clinical information obtained.

**Results:** Seventy-seven patients (age range 17-85; median age 51 yr and female to male ratio 5:1) met the search criteria. WL areas comprised from <5% to 100% of the lesions. 97% of patients had a background of chronic lymphocytic thyroiditis. 73% (56/77) showed classic WLV while 27% (21/77) showed WL areas admixed with TC areas (7 with WLV with <10 percent TC change at the periphery, 8 with variable mixture of WL and TC areas, and 4 with TCV with focal (often <10%) WL areas). The last 2 cases showed WL features, peripheral TCV and anaplastic carcinoma with extensive nodal as well as visceral metastases in one patient. In the 56 patients with pure WL, ipsilateral nodal metastases, extrathyroidal extension and vascular invasion were noted in 10 (18%), 8 (14%) and 2 (3.5%) patients respectively. Of the 21 patients whose tumors showed TC differentiation, 15 (71%) showed extrathyroidal extension,

14 (68%) had nodal metastases and 6 (29%) showed angioinvasion. One patient with TC features had nodal metastases with necrosis and solid growth consistent with poorly differentiated carcinoma.

**Conclusions:** Pure WLV-PTC is associated with an excellent outcome. Some of WLV-PTC also have TC differentiation. These patients have a higher incidence of extrathyroidal extension, lymph node metastases and angioinvasion. In addition, rare examples of WLV-PTC with TC areas may show dedifferentiation to anaplastic carcinoma. The findings in our series suggests that the relationship between WL and TCV of PTC deserves further study.

#### 1197 Immunohistochemistry of Nodular Fasciitis of the Head and Neck.

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**Background:** Nodular fasciitis (NF) is a reactive soft-tissue lesion that usually presents in the trunk and extremities but has also been described in the head and neck. Accurate diagnosis of NF is important to exclude malignancy, and is often possible based on age, body-site, clinical presentation, histology, and immunohistochemistry (classically vimentin+, SMA+, MSA+, S100-, desmin-). However, some cases remain difficult to distinguish from malignant lesions such as leiomyosarcoma, DFSP, low-grade myofibroblastic sarcoma, and Evans tumour, and a better characterization of NF protein expression could be helpful. To our knowledge, the published literature contains few cases evaluated for calponin (28/28 in one series), CD10 (1/3 in one series), and CD34 (0/10 in two series), and none for PGP 9.5, factor XIIIa, and CD56. This pilot study aims to investigate these less-well-characterized markers in NF of the head and neck.

**Design:** We searched the hospital database for cases diagnosed as NF from 1992 to 2010 and selected those originating in the head and neck. Original slides for these cases were reviewed, appropriate paraffin blocks were selected, and 7-micron sections were cut onto charged glass slides. Sections were stained for SMA, calponin, CD10, CD34, PGP 9.5, Factor XIIIa, and CD56. The presence (and compartment) or absence of staining was recorded.

**Results:** Six cases of NF fit our criteria. All were diagnosed by pathologists in subspecialty practice. Immunoreactivity is described in table 1.

Table 1. Immunohistochemistry Results

Antigen	Cases Staining Positive	Compartment
SMA	6 of 6	cytoplasmic
calponin	6 of 6	nuclear
CD10	6 of 6	cytoplasmic and membranous
PGP 9.5	6 of 6	nuclear and cytoplasmic
Factor XIIIa	3 of 6	nuclear and cytoplasmic
CD34	0 of 6	
CD56	0 of 6	

**Conclusions:** Based on this series of head and neck NF cases, SMA, calponin, CD10, and PGP 9.5 are consistently positive, Factor XIIIa is equivocal, and CD34 and CD56 are negative. These results confirm a well-established consensus for SMA, support initial findings for calponin and CD34, diverge from a previous result for CD10, and represent newly-reported findings for PGP 9.5, Factor XIIIa, and CD56. This immunoprofile sheds little new light on the histogenesis of NF. However, we propose that immunoreactivity for SMA, calponin, CD10, and PGP 9.5, combined with the traditional clinical and histologic features of NF, will be helpful in distinguishing NF from its mimicks.

#### 1198 Cancer/Testis (CT) Antigens and p53 Are Preferentially Expressed in High-Grade and Metastatic Mucoepidermoid Carcinoma.

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**Background:** CT antigens comprise a group of proteins that are normally only expressed in germ cells and yet are aberrantly activated in a wide variety of human cancers, making them attractive candidates for targeted cancer immunotherapy as well as potential diagnostic tumor markers. Higher frequency of CT antigen expression often correlates with tumor progression and with higher tumor grade, and this has been observed in metastatic melanoma, high-grade urothelial carcinoma and hormone receptor-negative breast cancer. The expression of CT antigens in salivary gland tumors, however, has not been studied.

**Design:** Protein expression of 6 CT antigens (MAGEA, NY-ESO-1, CT7, CT10, CT45 and GAGE) was immunohistochemically evaluated in tissue microarrays containing 137 pleomorphic adenomas, 41 Warthin's tumors, 8 basal cell adenomas, 33 mucoepidermoid carcinomas (MEC), 21 adenoid cystic carcinomas, 3 acinic cell carcinomas, and 2 carcinomas ex pleomorphic adenoma. Three tissue cores from each tumor were evaluated. Unequivocal nuclear staining in any tumor cells was considered positive. p53 overexpression was also evaluated and tumors with at least moderate staining in >20% of tumor cell nuclei were interpreted as positive.

**Results:** All benign salivary tumors were negative for the six CT antigens and p53 (0/185). In carcinomas, CT antigen was detected in 5/33 (15%) MEC and 2/21 adenoid cystic carcinomas; p53 staining was observed in 5 MEC and 1 carcinoma ex pleomorphic adenoma. Of 33 MEC in the series, 14 were either histologically high-grade or had evidence of metastasis, and 6 (43%) of these were positive for CT antigen (2 cases), p53 (2 cases) or both (2 cases), the latest subgroup including a case of brain metastasis that simultaneously expressed MAGEA, NY-ESO-1, GAGE and p53. In contrast, of the 19 MEC with low or intermediate grade and no evidence of metastasis, only 2 (10%) cases were positive for either p53 (1 case) or CT (1 case, NY-ESO-1 only). This difference is statistically significant ( $p = 0.047$ ).

**Conclusions:** Benign salivary gland tumors do not express CT antigens or p53. Carcinomas only infrequently express these antigens; however, high-grade and metastatic mucoepidermoid carcinomas showed preferential expression of CT antigens

and p53. This finding, although preliminary due to sample size, suggests CT antigens and p53 as potential markers for aggressive clinical behavior in mucoepidermoid carcinoma. Additional studies to confirm this finding are ongoing.

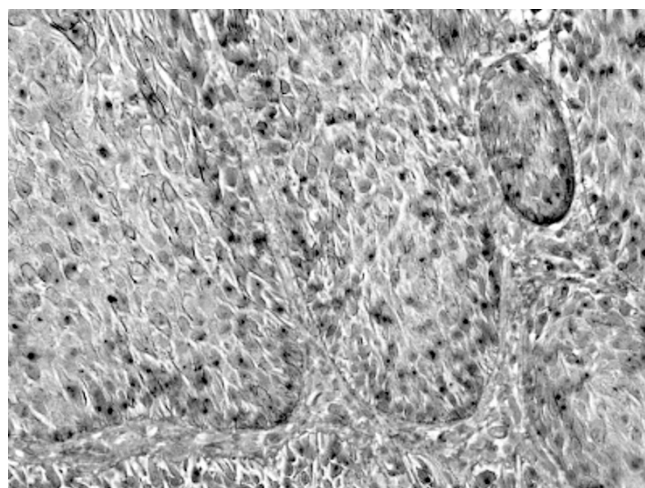
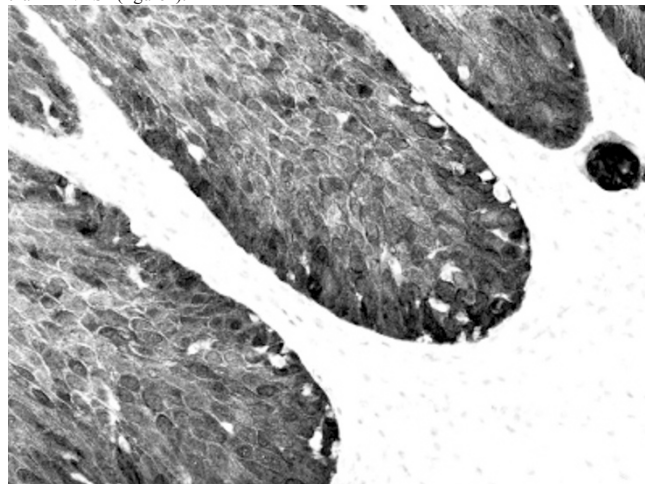
#### 1199 Is p16 Better and More Cost Effective Than HPV ISH for Detection of HPV-Associated Head and Neck Squamous Cell Carcinoma?

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**Background:** HPV-associated squamous cell carcinoma of the head and neck (HNSCC) has been identified as a distinct clinical and biological entity when compared to its non-HPV counterparts. Clinically, this subset of tumors has been shown to respond more favorably to chemotherapy and radiation, making their identification of paramount importance. Various detection methods including PCR, DNA in-situ hybridization (ISH) and p16 IHC are being used to determine the HPV tumor status. This study aims to determine the usefulness of p16 as a first-line marker for identification of HPV-associated HNSCC.

**Design:** 37 consecutively diagnosed HNSCC from 2009-2010 were reviewed with demographic data, lesional site, histologic subtypes, and tumor grade documented. All cases were simultaneously stained with p16 antibody (CINtec, Westborough, MA) and HR-HPV DNA ISH using HPV III INFORM probe (Ventana, Tuscan, AZ), which is a send-out test in our laboratory. The staining reactions of both techniques were analyzed and correlated with clinicopathological parameters.

**Results:** 32 cases had complete data and staining results. 9/32 (28.1%) and 16/32 (50.0%) were positive with HR-HPV ISH and p16 IHC, respectively, with HPV-/p16+ (7 cases), HPV+/p16+ (9 cases) and HPV-/p16- (16 cases). No cases stained HPV+/p16-. 7/16 (43.8%) p16+ cases were basaloid subtype, while all p16+ cases were located in the tonsil or oropharyngeal region. p16 staining (nuclear & cytoplasmic) (figure1) was more intense, easier to detect and interpret, more sensitive, and cheaper than HPV-ISH (figure2).



**Conclusions:** HPV-/p16+ cases may indicate HPV-associated tumors related to HPV subtypes not identified by the ISH probe or HPV unrelated mutational Rb gene inactivation. Conversely, HPV+/p16- cases may be due to inactive infection. Importantly, p16 IHC was easier to interpret, more sensitive and cost-effective than HPV-ISH in HNSCC, and may be able to discriminate active from latent HPV infection.

#### 1200 A Comparative Analysis of SOX2 Amplification in Squamous Cell Carcinomas of Lungs and Head and Neck.

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**Background:** SOX2 (sex-determining region Y-box 2) has been recently identified as a major oncogene that is amplified and activated in squamous cell carcinoma (SCC).

Several reports have indicated its role in early carcinogenesis of lung squamous cell carcinoma (LSCC). However, there is paucity of literature on SOX2 overexpression in head and neck squamous cell carcinomas (HNSCC). We have compared SOX2 expression in LSCC and HNSCC.

**Design:** 46 surgically resected, formalin fixed, paraffin embedded SCC (19 LSCC and 27 HNSCC) were randomly selected for SOX2 FISH analysis. Dual color FISH was performed using a Spectrum Green- labeled probe RP11-286G5 (3p24.3) (CHORI, Oakland, CA) and a Spectrum-Orange labeled, probe RP11-43F17 (3q26.3) (CHORI, Oakland, CA). At least 60 cells were scored for each case and control. A RP11-43F17 / RP11-286G5 signal ratio of 2.0 or greater was considered positive for SOX2 amplification. Results were then correlated with clinicopathologic parameters.

**Results:** LSCC more frequently showed SOX2 amplification (16/19, 84.2%) than HNSCC (10/27, 37%) (Fisher exact p = 0.002). While SOX2 non amplified LSCC had no disease related deaths, this difference in disease specific survival was not significant (log-rank p = 0.510). SOX2 amplification did not correlate with T stage or nodal status in LSCC. Although there was no statistically significant difference in frequency of SOX2 amplification between HNSCC with and without lymph node metastases, there was a trend towards more frequent SOX2 amplification in HNSCC with lymph node metastases. Specifically, SOX2 amplification was seen in 50% (6/12) node positive HNSCC patients, but not in node negative patients (p=0.1). Our results also suggest that frequency of SOX2 amplification of HNSCC may be influenced by anatomic location of the tumor. Of all HNSCC included in the study, oropharyngeal/hypopharyngeal tumors demonstrated the highest frequency of SOX2 amplification (5/9, 55%).

**Conclusions:** SOX2 amplification is a marker of squamous phenotype that is more frequently seen in LSCC than in HNSCC, though this cannot be used to distinguish site of origin. No significant outcome correlations were noted though there were small tendencies in both groups to have SOX2 amplification in more aggressive disease. The oropharynx/hypopharyngeal was the most commonly involved subsite in HNSCC.

#### 1201 P63 Expression Can Be Used in Differential Diagnosis of Salivary Gland Acinic Cell and Mucoepidermoid Carcinoma.

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**Background:** Differentiation of salivary gland acinic cell carcinoma from mucoepidermoid carcinoma can be diagnostically challenging as both may have prominent mucin production. P63 is a p53 homologue required for limb and epidermal morphogenesis. It is expressed in basal and myoepithelial cells of normal salivary gland tissues. In this immunohistochemical study, we examined the expression of p63 in salivary gland acinic cell and mucoepidermoid carcinomas and its use in differentiating these two entities.

**Design:** A search was performed and appropriate cases were selected from Lifespan Hospital System archives as well as the consult archives of one author (DG). 31 salivary gland acinic cell carcinomas (ACC) and 20 mucoepidermoid carcinomas (MEC) were examined for p63 expression by immunohistochemistry. The nuclear immunoreactivity was examined by both authors and was graded semi-quantitatively with negative being  $\leq 5\%$  cells staining, weak being  $>5$  to  $<25\%$  of cells staining, and strong being  $\geq 25\%$  of cells staining.

**Results:** Negative nuclear staining was seen in 30/31 (96.8%) of salivary gland ACC while 1/31 (3.2%) showed prominent nuclear staining. Strong positive nuclear staining was seen in 19/20 (95%) of salivary gland MEC cases while 1/20 (5%) cases showed minimal multifocal staining.

**Conclusions:** P63 is an immunohistochemical stain that can potentially aid in differentiating unusual acinic cell carcinomas with prominent mucin production from mucoepidermoid carcinomas of the salivary gland. According to this study, acinic cell carcinoma is almost always negative for p63 immunoreactivity while mucoepidermoid carcinoma is almost always strongly positive.

#### 1202 Anaplastic Nuclei and Tumor Cell Multinucleation Are Strong Predictors of Poorer Survival in Nonkeratinizing Oropharyngeal Squamous Cell Carcinoma.

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**Background:** Oropharyngeal squamous cell carcinoma (SCC) is frequently human papillomavirus (HPV) related. These tumors have a better prognosis, express p16, and frequently have a nonkeratinizing (NK) morphology. We have observed focal or diffuse nuclear anaplasia and/or multinucleation in NKSCC. Our aim was to determine if these features correlate with outcome.

**Design:** From a database of oropharyngeal SCC for which histologic typing (according to our established system) and p16 status were previously determined, we selected all surgically resected NK and hybrid SCC (excluded keratinizing SCC). Anaplasia was defined as any 40X field with  $\geq 3$  nuclei with diameters of  $\geq 5$  lymphocyte nuclei ( $\geq 30$  microns). Multinucleation was defined as any 40X field with  $\geq 3$  cells with multiple nuclei.

**Results:** Of the 125 cases, 121 (96.8%) were p16 positive. 94 were NKSCC and 31 hybrid SCC. 49 (39.2%) showed anaplasia and 54 (43.2%) multinucleation. Anaplasia and multinucleation were highly related ( $p < 0.0001$ ). Neither was correlated with any of the other major variables. Average follow up was 3.9 years, and 25 patients had died at the end of the study period. In univariate analysis, cases with anaplasia had worse overall and disease specific survival ( $p = 0.076$  and  $0.004$ , respectively). Multinucleation cases had worse overall, disease free, and disease specific survival ( $p = 0.034$ ,  $0.031$  and  $0.007$ , respectively). Higher T-stage correlated with worse overall, disease free, and disease specific survival ( $p = 0.0077$ ,  $0.0082$ , and  $0.010$ , respectively). No other variables correlated with survival including histologic type, smoking, N-stage, overall stage, sex, and margins (p16 not analyzed due to too few negative cases). In multivariate

analysis, there was worse 5 year overall, disease free, and disease specific survival with anaplasia ( $p=0.028, 0.66, \text{ and } 0.024$ , respectively) and with multinucleation ( $p=0.049, 0.055, \text{ and } 0.049$ , respectively).

**Conclusions:** Among the best prognosis group of oropharyngeal SCC (nonkeratinizing types and p16 expressing) which are surgically resectable, anaplastic and multinucleated histologic features independently predict poorer outcomes.

### 1203 Association of Cell Signalling Factors with Survival in HNSCC Treated with Chemotherapy or Radiotherapy Plus Cetuximab.

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**Background:** Head and neck squamous cell carcinomas (HNSCC) have an overall survival rate of 45% over the last three decades. Treatment is based on platinum or radiotherapy and recently, monoclonal antibodies anti EGFR. The aim of this study was to analyze the expression of cell signalling factors in both normal mucosa and tumor and their correlation with relapses or survival in patients treated with combination of cisplatin or radiotherapy with cetuximab.

**Design:** 41 patients treated with either cisplatin or radiotherapy plus cetuximab were included in this study. Tumors were located in larynx (13), pharynx (5) or mouth (23). Immunohistochemistry for pMAPK, pmTOR, pS6, 4E-BP1, p4EBP1, eIF4E, pEIF4E, Ki67 and p16 was performed. Expression levels were semiquantitatively evaluated as percentage and intensity of stained tumor cells and adjacent mucosa [H score]. Statistical analysis was made by means of cross-tabulation by Chi-square and Kruskal-Wallis statistical tests.

**Results:** In the analysis of cell signalling factors in the adjacent mucosa, an increase of p4E-BP1 (0.05) and eIF4E (0.06) and a low level of pmTOR (0.04) was associated with relapses or dead. Regarding the tumors, high levels of 4E-BP1 (0.01) associated with size of the tumors and high levels of eIF4E (0.05) or pEIF4E (0.03) with poorly differentiated carcinomas. A decrease of PTEN expression (0.05), pmTOR (0.05) or low Ki67 (0.03) or p16 (0.005) were associated with relapses or dead. Importantly, the association between low levels of pmTOR or p16 and prognosis was only observed in patients treated with radiotherapy and cisplatin. No other significant correlations were observed in this study.

**Conclusions:** In the present series of 41 patients with HNSCC, expressions of p4E-BP1, pmTOR and eIF4E in the adjacent mucosa were predictive factors of worse prognosis showing significant correlation with survival. Lack of PTEN expression in tumor cells was the most significant factor associated with relapses or survival. Low levels of pmTOR or p16 protein associate with less response to radiotherapy treatment. Ongoing studies analyze the relevance of cetuximab treatment in these therapeutical regimens to underline the role of cell signalling factors as predictive of response to these new therapeutical approaches.

### 1204 High Risk Human Papillomavirus Is Not a Common Etiologic Agent in Nasopharyngeal Carcinoma.

*AD Singhi, JA Califano, WH Westra.* The Johns Hopkins Hospital, Baltimore, MD.

**Background:** The human papillomavirus (HPV), a cause of squamous cell carcinomas of the oropharynx, has also been implicated as an etiologic agent in carcinomas of the nasopharynx. The purpose of this study was to determine the frequency of high risk HPV in carcinomas of the nasopharynx, and to assess p16 immunohistochemical staining as a method for distinguishing HPV-related from Epstein Barr virus (EBV)-related carcinomas involving the nasopharynx.

**Design:** We performed p16 immunohistochemistry and in-situ hybridization for EBV and high risk HPV on 45 carcinomas of the nasopharynx. For HPV-positive cases, imaging studies were reviewed in an attempt exclude secondary extension from the oropharynx.

**Results:** By in-situ hybridization, 34 (76%) carcinomas were EBV+/HPV-, 7 (16%) were EBV-/HPV-, and 4 (9%) were EBV-/HPV+. EBV was more likely to be detected in carcinomas from non-Caucasian than Caucasians (96% vs. 50%,  $p < 0.001$ ). Conversely, HPV was more likely to be detected in carcinomas from Caucasian than non-Caucasian (16% vs. 0%,  $p = 0.03$ ). Of the patients with HPV-positive carcinomas and available staging information, all 3 were found to have inferior extension into the palatine tonsil. All HPV-positive carcinomas were p16 positive by immunohistochemistry, but none of the HPV-negative carcinomas were p16 positive (100% vs. 0%,  $p < 0.001$ ).

**Conclusions:** High risk HPV can be detected in a subset of carcinomas involving the nasopharynx, but many if not all of these likely represent secondary extension from an oropharyngeal primary. P16 immunohistochemistry is a reliable marker for separating EBV-related from HPV-related carcinomas of Waldheyer's ring.

### 1205 Cribriform Adenocarcinoma of the Tongue: Characterization of a New Entity.

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**Background:** In 1999, we have described 8 cases of an unusual carcinoma of the tongue that resembled solid and follicular variant of papillary carcinoma of the thyroid, and we coined the name "cribriform adenocarcinoma of tongue" (CAT). It is currently debated whether CAT is a distinctive tumor type or merely a morphological variant of polymorphous low-grade adenocarcinoma (PLGA). Here we present a new series of 21 tumors with histomorphological features of CAT in order to better characterize a poorly recognized entity.

**Design:** The 21 patients comprised 12 men and 9 women, with a mean age of 61 years (range 37-88). Most tumors presented with a mass in the tongue (13) or in the tonsils (2), followed by palate (4), and one each was located in the lip and retromolar buccal

mucosa. The mean size of the tumors was 2.6 cm (range 2.1 to 4.5 cm). In three patients, the primary site was unknown and first clinical manifestation was cervical lymph node metastasis. Together, 15 patients presented with cervical lymph node metastasis at the time of diagnosis. Clinical follow-up was available in 15 cases, and ranged from 1 to 13 years.

**Results:** Microscopically, the tumors have a lobulated growth pattern with intervening bands of compressed fibrovascular stroma. Interspersed in the solid parts areas are sheets with compressed tubulo-glandular, microcystic and cribriform structures. The bland looking vesicular nuclei are large with frequent small single nucleoli and considerable nuclear overlapping. The most striking cytological characteristics are pale-staining nuclei with ground-glass appearance resulting in resemblance to solid and follicular variant of papillary thyroid carcinoma.

**Conclusions:** The evidence favoring CAT as a separate tumor entity from PLGA can be derived from the facts that CAT originates almost exclusively in the base of tongue, it has a typical morphology and extensive clear or "ground glass" nuclear features. Furthermore PLGA shows a much more diverse range of architectural patterns than CAT. None of the described patients and other potential candidates in the literature have died of disease despite the fact that most presented with unilateral or bilateral neck lymph node metastases. It is likely that CAT is a tumor with early lymphotropic metastases into the regional lymph nodes, but in spite of this it has an excellent prognosis.

### 1206 FLI1 and CD57 Expression in Round Cell Sinonasal Tumors.

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**Background:** Round cell sinonasal tumors (RCSNT) are rare and can be difficult to differentiate and classify. Friend leukemia integration 1 (FLI1) is a nuclear transcription factor expressed in normal and neoplastic endothelial cells, Ewing's sarcoma/primitive neuroectodermal tumor family (EWS/PNET), neuroendocrine carcinoma and malignant melanoma (MM). CD57 (Leu7) is a myelin-associated glycoprotein expressed in natural killer, schwannian and certain neuroendocrine cells and their corresponding neoplasms. We aim to assess FLI1 and CD57 expression in RCSNT and their potential diagnostic utility.

**Design:** We performed a database search for malignant RCSNTs from 1995 to 2010. All cases were reviewed and diagnoses were updated to current WHO classification. All tumors with additional material available were stained with CD57 (NK1, Neomarker, Fremont, CA, 1:100) and monoclonal FLI1 (G146-222, Biocare Medical, Concord, CA, 1:100). Reaction in more than 10% positive cells was considered positive. FISH using EWSR1 (22q12) Dual Color, Break Apart Rearrangement Probe (EWSR1 Break Apart, Abbott Molecular Inc, Des Plaines, IL) was performed in selected cases.

**Results:** 49 patients with RCSNTs were identified, 24 with available material were included as follows: 12 olfactory neuroblastoma (ONB), 2 sinonasal undifferentiated carcinoma (SNUC), 6 mucosal malignant melanoma (MMM), 3 rhabdomyosarcoma (RMS) and 1 Schneiderian carcinoma (SC). Original diagnoses were revised in 4 cases: 2 SNUC were reclassified as SC and ONB and 1 poorly differentiated neuroendocrine carcinoma and 1 PNET/EWS as ONB. Immunohistochemistry results are presented in table 1. FLI1 expression was common in MMM and had weak to moderate intensity in ONB. CD57 was diffusely expressed in most ONB, staining both tumor cells nests and surrounding sustentacular/schwannian cells. EWS gene rearrangement was not identified in any of the 4 FLI1-positive cases tested (3 ONB, 1 SNUC).

**Conclusions:** Monoclonal FLI1 expression does not correlate with EWS rearrangement and has little discriminating value among various RCSNTs. CD57 is strongly and diffusely expressed in ONB and may be useful in the differential diagnosis in selected cases.

FLI1 and CD57 expression in RCSNT

	ONB (%)	SNUC (%)	MMM (%)	RMS (%)	SC (%)
FLI1	4/12(33)	1/2(50)	5/6(83)	0/3(0)	0/1(0)
CD57	11/12(92)	1/2(50)	0/6(0)	3/3(100)	0/1(0)

### 1207 High-Risk HPV E6/E7 mRNA Detection by a Novel In-Situ Hybridization Assay Strongly Correlates with p16 Expression and Patient Outcomes in Oropharyngeal Squamous Cell Carcinoma.

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**Background:** The association of human papillomavirus (HPV) with oropharyngeal squamous cell carcinomas (OSCC) has been well established. HPV has been detected in 60-80% of these tumors by DNA-based in-situ hybridization (DNA ISH) and/or PCR. Though these methods detect the presence of HPV, they do not provide information on active viral transcription. HPV E6/E7 mRNA is considered by many to be the best indicator of tumor HPV status, but its detection in situ has not been demonstrated. We utilized a novel RNA in-situ hybridization (RNA ISH) assay called RNAscope to detect HPV E6/E7 mRNA in OSCC and correlated the results with DNA ISH, p16 expression, and patient outcomes.

**Design:** We constructed tissue microarrays (TMAs) of OSCC tumors for which p16 immunohistochemistry and HPV DNA ISH were previously performed. These TMAs were used to detect E6/E7 mRNA of HPV-16 and other high risk (18,31,33,35,52, and 58) HPV types in tumor cells by a chromogenic RNAscope assay. Staining was blindly reviewed and scored for the presence of HPV E6/E7 mRNA as positive or negative. P16 immunohistochemistry was classified as positive or negative: 95% of the cases were either strongly or diffusely positive or were completely negative. HPV DNA was considered positive for any definitive nuclear staining in tumor cells.

**Results:** There were 192 cases. HPV E6/E7 mRNA was detected in 154 patients by RNA ISH (80.2%) and p16 was positive in 146 (76.0%). Concordance of p16 and HPV E6/E7 mRNA, p16 and HPV DNA, and HPV E6/E7 mRNA and HPV DNA was 95%, 82%, and 81%, respectively. Only one case was p16-positive, HPV E6/E7 mRNA-

negative, and only 9 cases were p16-negative, HPV E6/E7-positive. Overall survival strongly correlated with HPV E6/E7 mRNA (HR= 0.24,  $p=9.59E-08$ ), HPV DNA (HR= 0.36,  $p=2.23E-04$ ), and p16 (HR= 0.24,  $p=1.93E-07$ ). Disease-specific survival also strongly correlated with HPV E6/E7 mRNA expression (HR= 0.25,  $p=3.76E-04$ ), HPV DNA (HR= 0.25,  $p=5.29E-04$ ), and p16 (HR= 0.20,  $p=1.39E-05$ ). The clinical significance of HPV E6/E7 mRNA-positive, p16-negative cases could not be defined due to the small number of cases.

**Conclusions:** RNA ISH is significantly more sensitive than DNA ISH in detecting HPV in OSCC. There is a very tight correlation between p16 and HPV E6/E7 mRNA, such that both were comparable in stratifying patient outcomes and both doing so more strongly than DNA ISH.

### 1208 High-Throughput Analysis of 79 Cases of Follicular Tumors of the Thyroid.

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**Background:** Although fine needle aspiration cytology (FNAC) is a very efficient method for diagnosing thyroid nodules, distinction between follicular adenomas and carcinomas may still represent a challenge. Identification of distinct genomic alterations between these two entities, potentially applicable to FNAC specimens by fluorescent in situ hybridization (FISH), would be therefore of major interest.

**Design:** We have studied 79 surgically excised and histologically verified frozen thyroid samples consisting of 33 cases of follicular adenomas and 46 cases of follicular carcinomas by comparative genomic hybridization (aCGH) on 244K arrays.

**Results:** Of the 79 patients, 44 were female and 35 were male with a median age of 56 (21-64) and 58 (28-87) years, respectively. Mean size of follicular adenomas was 3 (1-6.4) cm and histological subtypes were microfollicular in 7 (21%) cases, macrofollicular in 8 (24%) cases and mixed subtype in 18 (55%) cases. Mean size of follicular carcinomas was 3.7 (1-15) cm and histological subtypes were microinvasive in 28 (61%) cases and widely invasive in 18 (39%) cases. Differential analysis on the respective aCGH profiles by T-test showed that 89 differential aberrant regions were observed at a p-value threshold of 0.005 between carcinomas and adenomas, including the following genes of interest: MAGEA clusters, CDK16, FGF13, among others. aCGH also disclosed 3 different large-scale genomic abnormalities which were only specifically observed in a subpopulation of follicular carcinomas: gain of chrX (11 cases, 24%), loss of chr22 (11 cases, 24%) and loss of chr1p (8 cases, 17%). In most cases, these abnormalities were mutually exclusive, giving an overall coverage of 52% of carcinomas. Further analysis refined the identification of carcinomas specific subregions: loss on 1p35.3 (10 cases, 22%), specifically targeting the tumor suppressor gene EPB41, loss on 22q11.23 (16 cases, 35%), and gain on Xq28 (15 cases, 33%), containing a MAGEA cluster and the CETN2 gene involved in cell cycle and nucleotide excision repair pathways. Combination of these 3 subregions allowed the identification of 29 out of the 46 carcinomas (63%). In addition, anomalies of distinct chromosomal regions were significantly associated with metastatic status and overall survival.

**Conclusions:** We have identified a series of genomic abnormalities of diagnostic and prognostic interest and potentially applicable using FISH on FNAC material from thyroid samples.

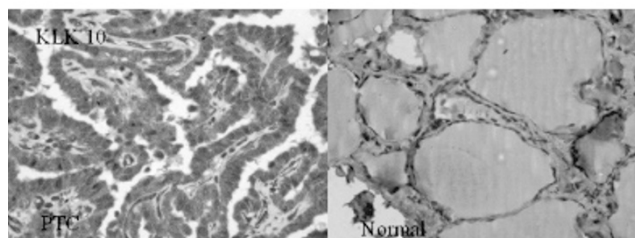
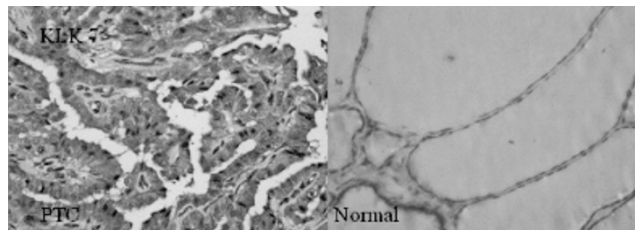
### 1209 Differential Expression of Kallikrein 7 and Kallikrein 10 in Papillary Thyroid Carcinoma and Normal Thyroid Tissue.

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**Background:** Papillary thyroid carcinoma (PTC) is the most common thyroid malignancy and its incidence is rising. As definitive diagnosis before surgery is often difficult, efforts have been made to identify biomarkers to distinguish PTC from benign nodules.

**Design:** Total RNAs from PTC and matched, normal thyroid tissue from 7 patients were analyzed with GeneChip Human Genome U133 plus 2.0 Arrays. Scanned output files were analyzed using dChip v1.3 and Affymetrix MicroArray Suite 5.0 (MAS 5.0) software. Quantitative real time RT-PCR (qRT-PCR) and western blotting for Kallikrein 7 (KLK 7) and Kallikrein 10 (KLK 10) were performed using RNA and protein from the same patients as used for microarray. Paraffin-embedded sections from 17 cases of PTC with surrounding normal tissue and a tissue array slide including 22 cases of PTC and 27 normal thyroid tissues were stained with anti-KLK 7 and KLK 10 antibodies. The results were scored 0 to 4+ based on the immunohistochemistry (IHC) density.

**Results:** Microarray data indicated the differential expression of 177 genes in PTC. Compared to matched normal tissue, two of Kallikrein gene family members, KLK7 and KLK 10 are significantly overexpressed in all seven PTC samples, with a fold change of 24.72 ( $p<0.05$ ) and 15.66 ( $p<0.05$ ), respectively. These findings were validated by qRT-PCR with a fold change of 19.0 ( $p<0.05$ ) for KLK 7 and 31.63 ( $p<0.05$ ) for KLK 10, and western blotting. IHC scoring revealed that KLK 7 and KLK 10 are significantly higher ( $p<0.01$  for both) in PTC (2.78±1.12, 3.05±1.10, respectively) than those in normal thyroid tissue (1.33±0.67, 1.24±0.65, respectively). Representative PTC/normal tissue IHC for KLK 7 and KLK 10 are shown below.



**Conclusions:** Our initial study demonstrated that KLK7 and KLK 10 are significantly overexpressed in PTC, compared to normal thyroid tissue. These two proteins may be useful as diagnostic biomarkers for PTC, as well as for the differential diagnosis of FNA specimens. The role of these molecules in the molecular pathogenesis needs further evaluation.

## Hematopathology

### 1210 Follicular Lymphoma "In Situ" and with Partial Lymph Node Involvement Represent Genetically Early Stages of Follicular Lymphoma Development.

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**Background:** Follicular lymphoma (FL) is characterized, in most cases, by the t(14;18) translocation resulting in *BCL2* gene rearrangement. Secondary genetic alterations have been found in 70-90% of manifest FL (mFL), and are thought to play a role in the progression and/or transformation of the disease. Although most patients with FL have widespread disease at diagnosis, one third of them present with stage I-II disease, and often show only partial lymph node (LN) involvement by FL at diagnosis. Furthermore, cases with normal LN architecture and strong *BCL2*-expression in follicular center cells without any other evidence of disease have been designated as FL "in situ" (FLIS). The biological significance of these findings is still unclear. The main aim of the study was to identify possible early secondary genetic events in the evolution of t(14;18) positive FL.

**Design:** Twelve cases of FL (5 pure FLIS, 3 "paired samples" of FLIS with their corresponding mFL, 3 FL with partial LN involvement and 1 FL with partial involvement and "in situ" component) were analyzed by oligonucleotide-based array CGH (244K arrays, Agilent Technologies) following microdissecting. The t(14;18) was evaluated with a *BCL2* break-apart probe by FISH. Clonality analysis of the *IGH* gene was performed in the 3 "paired samples".

**Results:** All FLIS cases showed a *BCL2* break by FISH indicative of the presence of a t(14;18). *IGH* PCR analysis demonstrated that FLIS and their corresponding mFL were clonally related. CGH analysis did not detect secondary chromosomal imbalances in any FLIS or FL with partial LN involvement; however, known copy number variations (CNVs) were identified in all cases. The three mFL showed secondary genetic imbalances (e.g. -6q, -10q) frequently reported in FL. One of the mFL was negative for *BCL2* expression whereas the FLIS component was strongly positive. Both of them had a *BCL2* break by FISH, suggesting secondary alterations of the *BCL2* gene during the progression of the disease.

**Conclusions:** 1) FLIS and FL with partial lymph node involvement probably represent early stages of FL lymphomagenesis, as evidenced by the absence of secondary genetic alterations. 2) The FLIS were clonally related to the syn-/metachronous mFL. 3) Array CGH identified secondary genetic aberrations in mFL, suggesting that additional genetic alterations are needed to progress from FLIS to manifest FL.

### 1211 Thyroid Lymphomas Express Similar Immunoglobulin VH Genes.

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**Background:** Thyroid extranodal marginal zone B-cell lymphomas of MALT type (MALT lymphomas) appear to develop from reactive infiltrates associated with Hashimoto's thyroiditis but have not been as well studied as other MALT lymphomas. Whether the more common thyroid diffuse large B-cell lymphomas (DLBCL) typically represent transformation of previously undetected MALT lymphomas is also unknown. Thyroid lymphoma immunoglobulin heavy chain variable (VH) genes were analyzed to evaluate whether DLBCL and MALT lymphomas show features suggestive of relatedness and/or growth stimulation by common antigens.