2. IMP2 staining differentiates serous from endometrioid carcinomas:

Figure 2. IMP2 differentiates serous from endometrioid carcinoma

Pathology diagnosis	IMP2 staining pattern				
# of cases	<5%	<25%	<50%	<90%	>90%
Benign: 93	0	0	0	0	93
Endometrioid					
FIGO-1: 89	54	27	8	0	0
FIGO-2: 57	3	28	26	0	0
FIGO-3: 32	0	8	21	3	0
Serous: 27	0	0	0	0	27
Mixed: 22					
Endomotrioid: 22	0	16	5	1	0
Serous: 22	0	0	0	0	22

Conclusions: Our study indicates serous carcinomas are diffusely and strongly IMP2 positive. Endometrioid carcinomas however are always partially positive or negative. IMP2 staining pattern therefore can differentiate these two carcinomas.

1214 Cytological Results and Clinical Findings Associated with 265 Histopathological Diagnoses of Cervical Glandular Neoplasia: Results of 10 Years

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Background: The effects of population screening on cervical glandular neoplasia (CGN) have been limited. The incidence of invasive cervical adenocarcinoma (ICA) has continued to increase during the same period an accelerated decline in cervical squamous carcinoma has been documented, both trends coinciding with the widespread introduction of liquid-based cytology.

Design: A computer-based search of our databases was conducted over a study period of almost 10 years between January 2000 and September 2009 to identify the cases diagnosed surgically as cervical AIS, ICA, or invasive cervical adenosquamous carcinoma. Pathological findings, clinical history, and previous Pap test results were documented.

Results: 265 patients were identified with cervical glandular neoplasia from our databases, including 81 cases of ICA, 17 cases of invasive adenosquamous carcinoma, and 167 cases of AIS. Of these cases, 60% had associated CIN (126 CIN2/3, 34 CIN1).Among 98 women with invasive carcinoma, 85 (86.7%) had associated AIS in histology. The Pap tests or clinical findings leading directly to histologc diagnoses of CGN included 105 (39.6%) AGC/AEC/AIS, 35 (13.2%) HSIL, 16 (6.0%) LSIL, 29 ASC (10.9%), 20 (7.6%) AGC/SIL, 8 (3.0%)AGC/ASC-H, 44 (16.6%) unknown, and 9 (3.4%) with clinical symptom/sign. 23 patients with CIN2/3 cervical biopsy results had AIS diagnosed on subsequent LEEP/Cone biopsies. 14 women with a Pap test history of AGC had initially negative ECC follow-up, not accompanied by cervical biopsies, resulting in delayed diagnoses of CGN. High percentage of women had abnormal and normal Pap test history.

Pap Test History Preceding Abnormal Pap Tests Directly Resulting in Histopathological Diagnoses of Cervical Glandular Neoplasia

Time interval preceding histologic dx	No. Patients	At least one abnormal Pap test (%)	At least one normal Pap test (%)	Both normal and abnormal Pap tests (%)	AGC Pap test (%)
0-1 yr	80	26 (32.5)	54 (67.5)	0	11 (13.8)
>1-3 yr	114	41 (36.0)	96 (84.2)	23 (20.2)	18 (15.8)
>3-5 yr	121	46 (38)	109 (90.1)	34 (28.1)	20 (16.5)

Conclusions: Early diagnosis of cervical glandular neoplasia remains challenging. Preceding negative cytological and histologic results reflect the special challenges associated with both endocervical cytological sampling and with identification and sampling of CGN during colposcopic examinations. Women with AGC Pap should remain classified as high risk patients even after negative/benign histologic follow-up results.

Head & Neck

1215 Salivary Mucoepidermoid Carcinoma: Clinicopathologic Study of 53 Patients

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Background: Mucoepidermoid carcinoma (MEC) is one of the most common salivary malignancies. We previously published a grading system which modified the AFIP schema by adding the variables of tumor pattern of invasion, lymphovascular invasion, bony invasion and increasing the weighted value for perineural invasion. Our goal is to 1) review the clinicopathologic features of a new cohort and 2) compare the two grading systems.

Design: We searched MMC pathology files (1978-2009) and reviewed cases diagnosed as "MEC". Tumors were graded according to both published criteria. Charts were reviewed for tumor site, stage, treatment, and outcome.

Results: We reviewed 67 tumors; 14 were excluded after reclassification as squamous carcinoma (SCC) (3), high-grade salivary duct carcinoma (HGSDC) (3), low-grade salivary duct carcinoma (1), carcinoma-ex-BMT (1), sebaceous carcinoma (1), carcinoma-not-otherwise-specified (3), cystadenoma (1), and MEC metaplasia in BMT (1). The diagnosis of MEC was confirmed on 53 tumors. Ages ranged from 18-77 (mean 50), female: male ratio was 3:1. Most common sites were parotid (55%), oral cavity (32%), and submandibular gland (9%). Unusual presentations in 2 patients merit mention. One grade I MEC was diagnosed as a benign cyst. This tumor was inadeqately excised and its persistence became manifest 9 years later. This woman is disease-free 3 years after definitive surgery. One woman with grade II parotid MEC presented with a draining cutaneous fistula. The frequencies of grades I, II, & III MEC by our criteria are 36%, 34%, & 30%, respectively. Our criteria upgraded 30% of MEC: 20% from grades I to II, 8% from grades II to III, and 2% from grades I to III. Information on outcome was available on 35 patients. Two patients (grade III, both schemas) died of disease at 12 and 16 months, respectively. One patient is alive with persistent disease. Thirty two patients are disease-free (mean 44 months).

Conclusions: MEC is usually associated with good outcome when appropriately treated. The diagnosis of MEC is unlikely for tumors with extreme pleomorphism, abundant keratinization, or hyalinization. SCC and HGSDC comprised the most common tumors misdiagnosed as MEC. Our proposed grading criteria upgraded 30% of MEC compared to the AFIP criteria, however the limited number of disease-progression events did not allow for comparison of the performance of the two grading classifications.

1216 Expression of the GLUT1 Glucose Transporter in Adenoid Cystic Carcinomas Transformed into Adenocarcinoma or Undifferentiated Carcinoma

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Background: In neoplasia, enhanced expression of GLUT1 has been interpreted as increased glucose uptake as well as response to tissue hypoxia. The latter has been implicated in the pathogenesis of dedifferentiated phenotype in certain types of cancer. GLUT1 expression has also been reported to correlate with poor prognosis, tumor aggressiveness and lymph node metastases. Transformation of adenoid cystic carcinoma to adenocarcinoma or undifferentiated carcinoma (ACC-Ad/UC) has been linked to accelerated clinical course and high propensity for lymph node metastases. In order to analyze whether a) hypoxia could be involved in the pathogenesis of ACC-Ad/UC and b) GLUT1 expression could be used as a predictor of clinical outcome, we looked at the expression of this marker in conventional and transformed areas of ACC-Ad/UC.

Design: In six cases of ACC-Ad/UC and in 18 ordinary ACC the immunohistochemical expression of GLUT1 was assessed using a three-tiered scale: >10% - 25%, and > 25- 50% and >50% of positive cells. α-SMA was used for detection of myoepithelial cells and the proliferation index was evaluated by Ki-67. Demographic and clinical information was obtained from the patients' medical records.

Results: The transformed areas were classified according to histological patterns as adenocarcinoma in 4 cases and solid undifferentiated carcinoma in 2. Loss of myoepithelial layer was found only in the transformed component, which also showed higher Ki-67 index. In ACC-Ad/UC, only one patient died of disease and presented lymph node metastases. Three did not show recurrence (median follow-up 54 months), including one long-term survivor (131 months). Both conventional areas of ACC-Ad/UC and ordinary ACC were negative for GLUT1 in most cases (83.3% and 81.3%, respectively), whereas the Ad/UC component presented increased expression of GLUT1+ in 50% of cases. However, the degree of GLUT1 expression correlated neither with clinical outcome nor with the histological subtype of the transformed component.

Conclusions: The scanty expression of GLUT1 in conventional areas of ACC-Ad\UC as well as in ordinary ACC suggests that hypoxia may not play a crucial role in the development of the Ad/UC phenotype. The enhanced metabolic demand leading to increased glucose uptake could explain higher GLUT1 expression in the Ad/UC areas, but GLUT1 cannot be considered useful as prognostic marker.

1217 Does Tall Cell Histology Affect the Clinical Behavior of Papillary Thyroid Microcarcinoma?

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Background: Papillary thyroid microcarcinoma (PTMC) is an indolent tumor with favorable long term prognosis, however, recurrences in the neck and distant metastases have been reported. Tall cell variant of papillary thyroid carcinoma (TCV-PTC) is an aggressive variant of papillary thyroid, which can show increased propensity towards lymph node metastases, tumor recurrences and distant metastasis as compared to classic variant of papillary thyroid carcinoma. In this study we evaluated the effect of tall cell histology on the biologic behavior of PTMC.

Design: The computerized pathology files at our institution were searched for cases diagnosed as papillary thyroid carcinoma and TCV-PTC. Fourteen cases of PTMC with tall cell histology (PTMC-TCV) were identified. Clinicopathologic data and follow-up (serum thyroglobulin measurements, radiologic studies and/or additional tissue sampling) through present date were extracted from medical records and was compared with similar data from 10cases of PTMC classic variant (PTMC-CV) and 16 cases of PTMC follicular variant (PTMC-FV).

Results: PTMC-TCV presented as a single focus in 8 (57%) and as multiple foci in 6 (43%) cases(size range 0.2-1.0 cm), whereas PTMC-CV and PTMC-FV presented as single focus in 7 and 13 and as multiple foci in 3 cases each respectively (size range 0.2-1.0 cm). A higher rate of lymphovascular invasion(LVI), extrathyroidal extension (ETE) and lymph node metastases (LN-mets) was seen in PTMC-TCV as compared to PTMC-CV and PTMC-FV; LVI 28% vs. 10% and 6%, ETE 50% vs. 10% and 0% and LN-mets 28%% vs. 10% and 6%. Regional tumor recurrence was seen in 5 (5/14 36%) cases of PTMC-TCV, in 1 (1/10 10%) of PTMC-CV and in 1 (1/16-6%) case of PTMC-FV. Distant metastases were not seen in any three variants of PTMC at an average mean follow-up of 9-years.

Conclusions: In our experience, tall cell histology portends a more aggressive clinical course in PTMC cases. Therefore, this should be mentioned in the pathology report as these cases may require close clinical follow-up as compared to the cases of PTMC-CV and PTMC-FV.

1218 KIT, EGF-Receptor and HER2/Neu Expression in Malignant Melanomas of the Sinonasal Mucosa. Clues to a Specific Carcinogenic Pathway Different from Cutaneous Melanoma

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Background: KIT (CD117), EGFR (Epidermal Growth Factor Receptor) and HER2/neu, are trans-membrane receptor tyrosine kinases which are amplified and/or overexpressed in a variety of human neoplasms, including a subset of cutaneous melanomas. However, KIT expression in sinonasal melanomas, a rare site for that tumor, has been seldom studied. Moreover, EGFR and HER2/neu expression are totally unknown in these tumors.

Design: KIT, EGFR (Epidermal Growth Factor Receptor) and HER2/neu expression were studied in 18 cases, using immunohistochemistry. The grading of the immunostaining was performed on a sliding scale of 1+ to 3+ according to the percentage of reactive cells (0 = 0-5%; 1+= 6%-40%, 2+= 41%-70%; 3+ =71-100%). In addition, c-kit mutation analysis was performed in one of these cases.

Results: KIT overexpression was observed in 15 of the 18 cases (83 %). Immunohistochemistry for CD117 was weakly positive (1+) in one case (6%), moderately positive (2+) in 7 cases (47%), strongly positive (3+) in 7 cases (47%). CD117 immunoexpression persisted in the invasive component. The case tested for mutational analysis was positive for c-kit mutation within exon 17. Interestingly, only one (6%) of the 17 cases show a weak (1+) EGFR immunoreactivity which is in contrast to what is reported for cutaneous and uveal melanomas. No HER2/neu expression could be seen.

Conclusions: Our data indicate that sinonasal melanomas follow a specific carcinogenic pathway characterized by a lack of EGFR expression and a frequent and high level of KIT expression. That latter molecule can even carry activating mutation. Evaluation of the relevance of c-kit as molecular target in sinonasal melanoma warrants further investigation.

1219 HPV Detection in Head and Neck Squamous Carcinoma: A Comparison of Methods

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Background: Substantial evidence confirms a causal relationship between HPV and some HNSCC, most commonly oropharyngeal SCC and HPV16. HPV+HNSCC may have a better response to chemo-radiation and longer survival than HPV-HNSCC. Future treatments will be tailored based on HPV status and pathologists will be requested to make this determination. IHC for p16 and ISH with HPV DNA probes are touted as surrogates to PCR analyses. IHC is readily available, easily performed, and easily interpreted, although few reports address optimal cut-off values. In contrast, HPV ISH is not routinely available and interpretation is more difficult. It is important and timely to compare the sensitivity and specificity of commercial ISH/IHC tests to the gold-standard of HPV RNA PCR on frozen samples.

Design: Frozen tumors were tested by RT-PCR for HPV16 E6/E7 RNA. Tissue microarrays (TMA) were constructed from corresponding FFPE tumors and tested for p16 by IHC (BD Pharmingen antibody). Nuclear and cytoplasmic staining was quantified for intensity and % distribution; these values were incrementally correlated with HPV16 E6/E7 RNA status. The optimal combination of cut-off values was selected based on maximum area under the ROC curve. HPV ISH was assessed on these TMAs with two different probes: the INFORM^{R} HPV-III Fam16(B) probe (Ventana Medical Systems, Tucson, AZ) and HPV 16/18 probe (DakoCytomation, Carpinteria, CA).

Results: Sixty-eight tumors were studied; HPV16 E6/E7 was detected in **39%** by RT-PCR. The optimum cut-off values for p16+, as determined by ROC values, was **nuclear *plus* cytoplasmic staining ≥+2, ≥75%.** The **sensitivity** and **specificity** for p16 by IHC is **50%** (95% CI 30%, 70%) and **93%** (95% CI 81,96%), respectively, compared to RT-PCR for HPV16 E6/E7. We are continuing to study new cases by RT-PCR and IHC. Comparison of the ISH data (Ventana and Dako probes, read blinded by multiple observers) with the RT-PCR data is on-going.

Conclusions: The performance of p16 as assessed by IHC on TMAs, using the above cut-offs, indicates that this test has good specificity, but poor sensitivity. HNSCC with no p16 staining, or staining below these cut-off values, will still require additional HPV testing.

1220 Validation of the Risk Model in a New Patient Cohort with HNSCC

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Background: Some patients with low-stage (LS) HNSCC are expected to fail single modality therapy, indicating that more aggressive treatment would have been warranted. *Identifying LS patients at risk for disease progression (DP), for multimodality treatment, would represent an important advance.* Our Risk Model may aid in developing new treatment paradigms. Here we report the performance of our model in a new multi-center cohort.

Design: Eligible patients were identified and resection slides were reviewed (MBG). Kaplan-Meier (KM) analysis was performed for DP and OS, stratified by risk category. Stratified Cox proportional hazard regression was performed, adjusted for potential confounders.

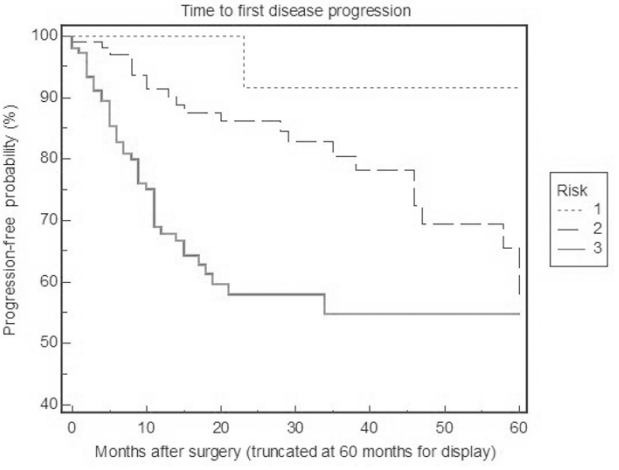
Results: Table 1 summarizes the cohort.

Table 1: Cohort data

	MMC	NYU	Manitoba
Number SCCs	225	30	56
Oral (%)	48	93	93
Oropharynx (%)	24	0	7
Larynx(%)	28	7	0
Age ⟨60 (%)	37	47	45
Low stage (%)	37	50	46
High stage (%)	63	50	54

311 primary SCC developed in 306 patients

Risk predicts time to DP ($p < 0.0001$) (Fig 1) and OS ($p < 0.0001$) by KM.



Results of the Cox multivariate regression analysis is shown in Table 2. Patients with high-risk SCC are more likely to progress early, compared to the remaining patients, after adjusting for confounders (HR 2.20 95% CI 1.14, 4.25).

Table 2: Risk & Disease Progression - Cox Model

	HR	95% CI	p
High-risk	2.20	(1.14, 4.25)	0.019
Female	0.80	(0.46, 1.41)	0.442
Age ⟩ 60	1.31	(0.77, 2.26)	0.323
Margin ∠ 5 mm	1.43	(0.78, 2.62)	0.242
Oropharynx	0.71	(0.31, 1.64)	0.425
Larynx	0.76	(0.35, 1.66)	0.490
T = 3/4	1.64	(0.89, 3.02)	0.112
LN+	1.01	(0.55, 1.87)	0.963
No adjuvant RT	1.37	(0.74, 2.54)	0.322

Larynx and oropharynx compared to oral cavity

Conclusions: We demonstrate the performance of the Risk Model in a new patient cohort, providing further evidence for using risk score to predict outcome in HNSCC patients.

1221 Undifferentiated Carcinoma of the Oropharynx Is an HPV-Related Tumor with a Good Prognosis

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Background: Undifferentiated carcinoma, also termed "undifferentiated carcinoma of the nasopharyngeal type" and "lymphoepithelioma," is an uncommon and histologically distinct tumor in the oropharynx, which, in Western countries, has been clearly shown not to harbor Epstein Barr virus. However, no study has analyzed these tumors for HPV.

Design: Oropharyngeal tumors with a diagnosis of "undifferentiated carcinoma" were retrieved from the department files from 1989 to 6/2009. After consensus review by all three study pathologists, sixteen were found to have the typical histologic features and to lack distinguishing characteristics of other oropharyngeal malignancies. Of these, 15 had material available for ancillary studies. Immunohistochemistry (IHC) for p16 and p53 and in-situ hybridization (ISH) for HPV were performed on these cases. Cases with positive p16 IHC, but negative HPV-ISH, were analyzed by PCR for high risk HPV types. The results were correlated with pathologic findings and clinical follow up.

Results: The average age was 59.2 years with an average follow up of 3.2 years. Ninety-one percent (91%) were smokers. The majority (13; 81%) were located in the tonsil, with 2 in the base of tongue (13%) and 1 in the soft palate (6%). Thirteen (81%) had nodal metastases. Fourteen of the 15 (93%) showed positive p16 IHC staining, but only 8 of these 14 (57%) were positive for HPV by ISH. PCR was positive in all 6 of these ISH-negative, p16-positive tumors, however, demonstrating that 14 of the 15 (93%) tumors were HPV positive. The p16 negative tumor was strongly p53 positive, as were four p16 positive tumors, and overall, 5 tumors (33%) had strong p53 staining. Three patients had disease recurrence (average 7 months) and two died of disease for an overall disease free survival of 87%. Two year overall and disease specific survival was 73% and 100%. HPV status did not correlate with outcome as most were positive. All three patients with disease recurrence were HPV positive.

Conclusions: The majority of oropharyngeal undifferentiated carcinomas are HPV-related and p16 positive with a low rate of p53 mutation. Disease specific survival is very good and is comparable to the best published rates for HPV-related oropharyngeal carcinomas.

1222 Extracapsular Extension in Oropharyngeal Squamous Cell Carcinoma: Only Soft Tissue Metastasis Predicts Disease Recurrence but Does so Independent of HPV Status

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Background: Nodal metastases in head and neck squamous cell carcinoma (SCC) are common and extracapsular extension (ECE) portends a worse prognosis. However, there are no histologic criteria for ECE, nor have there been studies on ECE specifically in human papilloma virus (HPV)-related SCC. This study quantifies the extent of ECE in oropharyngeal SCC with a clear grading system and a particular focus on HPV-related tumors.

Design: Surgically treated oropharyngeal SCC cases with nodal metastases were identified from the department files. All received post op radiation. ECE was graded as 0 (tumor cells within substance of node only), 1 (tumor filling subcapsular sinus with thickened capsule), 2 (tumor ≤1mm beyond capsule), 3 (tumor >1mm beyond capsule), or 4 (soft tissue metastasis;no residual nodal tissue or architecture). Grade was agreed upon by both study pathologists. In-situ hybridization for high-risk HPV and immunohistochemistry for p16 were performed.

Results: Of the 101 cases, there were 13 grade 0, 25 grade 1, 7 grade 2, 19 grade 3, and 37 grade 4 lymph node metastases. 86 had material for testing; 66.2% were HPV positive and 90.7% p16 positive. ECE grades did not correlate with nodal size(p=0.28) or p16 status(p=0.8). In particular, grade 4 ECE was not associated with nodal size(p=0.24) or p16 status(p=0.5). In follow up, 10 patients(9.8%) had disease recurrence with 1(0.9%) local, 4(3.9%) regional, and 7(6.9%) distant failures. In univariate analysis, survival was no different for ECE grade 0 or 1 (no grade 1 patients recurred) while grade 4 was associated with shorter overall(p=0.001), disease specific(p=0.001) and disease free(p=0.002) survival. Multivariate analysis showed, after adjusting for p16, patients with ECE grade 4 had higher risk of recurrence or death(HR=2.2, 95%CI: 0.9-5.4), but the association did not reach statistical significance(p=0.087). In addition, 6 of the 7 patients who developed distant metastases had grade 4 ECE(p=0.009), and all but one of these was p16 positive.

Conclusions: The impact of ECE is diminished in oropharyngeal SCC relative to the other head and neck sites. 62 patients had ECE ≥2, but only 9 (15%) of these had disease recurrence. However, grade 4 ECE/soft tissue metastasis remains a strong predictor to recurrence, particularly distant metastasis, independent of HPV status.

1223 Human Papillomavirus (HPV)-Related Squamous Cell Carcinoma of the Oropharynx in African-Americans and Caucasians: A Comparative Study

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Background: HPV-related squamous cell carcinoma (SCC) of the oropharynx is well-documented as a distinct subtype of SCC. Patients with HPV-related SCC have substantially better survival rates than those with HPV-negative tumors. Differences in the incidence of HPV-related SCC between Caucasians and African-Americans have not been well examined. The aim of this study is to explore the frequency of HPV-related SCC of the oropharynx in African-Americans and Caucasians and to evaluate patient outcome in these two groups.

Design: Cases of stage III and IV oropharyngeal SCC were identified from an IRB approved radiotherapy database from 2000 to 2007. All patients received either definitive intensity-modulated radiation therapy (IMRT) or surgery followed by postoperative IMRT. In situ hybridization (ISH) for high-risk (HR) HPV subtypes and immunohistochemistry for p16, a surrogate marker of HPV-related tumors, were performed.

Results: Of 174 patients, 148 (85%) were Caucasian and 26 (15%) were African-American. There were no differences in sex, age, adjuvant chemotherapy or length of follow-up by race. HPV –ISH and p16 positive SCCs were much more common in Caucasians (63.5% and 83.1% of tumors) than in African-Americans (11.5% and 34.6% of tumors) [p <0.0001]. Caucasians were also more likely to have received postoperative IMRT rather than definitive IMRT compared to African-Americans (p=0.0011). African-Americans also had a higher frequency of T3/T4 tumors (p=0.0322). Disease-free survival was significantly shorter for African-Americans (p=0.0254). In multivariate analysis, treatment type (p=0.0002) and HPV status (p=0.0027), but not race (p=0.9834), were significant factors contributing to disease-free survival.

Conclusions: The frequency of HPV-related SCC of the oropharynx is much higher in Caucasians than in African-Americans. African-Americans presented with higher T-stage tumors and were more likely to receive definitive IMRT. The shorter disease-free survival observed in African-Americans may be due to HPV status and factors related to choice of treatment modality.

1224 Benign Tumefactive Pathology of Voice: Does Anybody Really Know What Kind It Is?

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Background: Benign lesions of the vocal cord (VC) primarily related to vocal abuse, reflux and smoking are clinically classified as: 1) polyps (P) (uni or bilateral, exophytic or sessile, anterior VC); 2) nodules (N) (symmetric swellings, anterior-mid VC); 3) Reinke's edema (RE) (uni or bilateral swellings of the entire VC); 4) cysts (C) (discrete unilateral swellings with epithelial lining). Histologic examination of all lesions except cysts has shown similar and variable presence of edema, vascular proliferation, extracellular fibrin ("amyloid-like"), basement membrane thickening & epithelial hyperplasia. Treatment varies: N and RE are initially treated conservatively, while P and C are excised surgically. There is a a lack of consensus among clinicians & pathologists regarding the classification of these lesions, and there exist no universal histologic criteria. This study aims to elucidate the clinicopathologic correlation, or lack thereof, among these benign lesions of the VC.

Design: A 20 year retrospective search of the UCMC surgical pathology database for all such benign lesions of the VC yielded 78 cases. They were reviewed by one pathologist for the above 5 histologic parameters and classified as P, N, RE, C or other. Of these, 41 also had endoscopic & stroboscopic images reviewed and classified separately by 2 otolaryngologists.

Results: Among the 41 cases reviewed clinically and histologically: All 3 agreed in 25% of cases, mostly P. None agreed in 17%. One clinician and pathologist agreed in 43%. The pathologist disagreed with the diagnosis of both clinicians in 15%. The overall kappa statistic was 0.17 (0.08-0.24, 95% confidence interval). Histology in all cases showed a range of epithelial hyperplasia, basement membrane thickening, edema, vascular proliferation, and fibrin/amyloid-like material. There were no histologic features that reliably distinguished among P, N or RE. C were all epithelial-lined. Stromal cell atypia was isolated. No suspicion of malignancy was identified.

Conclusions: Indefinite clinical and histologic criteria in these benign VC lesions may explain the poor agreement between the 2 clinicians and the poor clinicopathologic correlation. Only true cysts, having a lining, are histologically distinct. While treatment may be individualized per clinical judgement, the classification of a lesion as P, N or RE is neither clinically reproducible nor histologically unique.

1225 p16 Expression in Squamous Cell Carcinomas from Non-Cervical Primary Sites: An Immunohistochemical Study of 100 Cases

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Background: p16 is a cell-cycle regulatory protein that is upregulated in cells infected with oncogenic subtypes of human papillomavirus (HPV). Recent studies have suggested a role for p16 immunohistochemistry in determining the site of origin in cases of squamous cell carcinoma (SQCCA) of unknown primary. The aim of this study is to determine the incidence of p16 expression in SQCCAs from various sites (other than cervix, where most SQCCA are known to express p16) in order to assess the utility of p16 immunohistochemistry in determining the primary site of origin of cases of metastatic SQCCA.

Design: 100 cases of SQCCAs from 5 major primary sites including 20 each from lung, esophagus, tonsil, non-tonsillar head and neck and skin were retrieved from our surgical pathology archives. The histological features were reviewed. p16 immunohistochemistry was performed on a representative block from each case. Cases were considered positive if strong nuclear and cytoplasmic staining was detected in more than 10% of cells.

Results: p16 expression was detected in 47/100 (47%) cases overall, and was found in all primary sites tested, including tonsil (15/20, 75%), skin (9/20, 45%), non-tonsillar head and neck sites (8/20, 40%), lung (8/20, 40%), and esophagus (7/20, 35%). Of the non-tonsillar head and neck sites, the highest proportion of positive cases was from the nasopharynx (4/5, 80%). Most positive cases expressed p16 in greater than 90% of cells (36/47), with fewer cases showing expression in 50-90% (6/47) or 10-50% (5/47) of cells. Proportion of cells staining did not correlate with primary site or with degree of differentiation of the tumor.

Conclusions: p16 is expressed in SQCCAs from a wider variety of primary sites than generally appreciated. In addition to tonsillar SQCCAs, a large proportion of which are known to express p16, SQCCAs of the lung, esophagus and non-tonsillar head and neck sites commonly express p16. p16 expression therefore cannot be reliably used to establish the site of origin. Our findings additionally raise the possibility of HPV infection in the pathogenesis of SQCCAs of a wide variety of sites. Further investigation is needed to determine the correlation between p16 expression and HPV infection in these sites.

1226 Evidence of Submucosal Nerve Hypertrophy in Patients with Laryngomalacia

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Background: Laryngomalacia is a common cause of stridor in newborns. The underlying defect that causes laryngomalacia remains unknown; however, mechanisms involving inflammation and neuromuscular weakness have been proposed. The aim of this study is to evaluate the specific difference in diameter and surface area of submucosal nerves in laryngomalacia patients versus control subjects along with other stromal changes commonly observed in these patients.

Design: Patients with laryngomalacia in the period from January 2008 until August 2009 were collected from the files of a Children's Hospital. Laryngeal tissues from autopsy cases were used as a control group. Demographic data were collected from pathology reports. Each slide is scanned, the largest submucosal nerve spotted, image captured, digitized and measured for area and perimeter. Stromal abnormalities including myxoid degeneration, edema and inflammatory infiltrate were also evaluated.

Results: Supra-arytenoid tissue from 43 patients with laryngomalacia (25 males and 18 females) (age: median 0.41 years; range: 0.02-3.19 years) and 13 controls (12 males and 1 female) (age: median 0.5 years; range: 0.07-1.41 years) were examined. Both groups were matched for age (P=0.67). There was marked submucosal nerve hyperplasia in patients with laryngomalacia compared to controls group. In the patients group, nerve perimeter (median: 1765 μ; range 452-3877 μ) was higher than in the control group (median: 948.7 μ; range 413-3253.3 μ); this difference was statistically significant (P=0.002). In addition nerve surface (median: 182905 μ^2; range 13938-961007 μ^2) was significantly higher than in the control group (median: 60405 μ^2; range 9409-726521 μ^2[1] P=0.02). Furthermore, there was increased incidence of stromal abnormalities such as edema, submucosal myxoid degeneration, and lymphocytic infiltrate in the patients group.

Conclusions: In this study, we demonstrate the presence of significant submucosal nerve hyperplasia in children with laryngomalacia compared to controls. In addition,

these patients showed an increased incidence of stromal abnormalities. The cause-effect relationship between nerve and stromal changes and laryngomalacia is still unknown. However, our results suggest that laryngomalacia is related, at least in part, to an underlying neural abnormality.

1227 The Value of Frozen Section Diagnosis in Thyroid Pathology

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Background: Intraoperative frozen section (FS) for thyroid specimens is a useful tool to the surgeon, although its reliability to detect malignant lesions is highly variable. To compare the sensitivity of frozen section for diagnosis of thyroid pathology we studied our experience in a teaching hospital.

Design: The consecutive records of 369 patients who underwent thyroid surgery during a five year period (2004 to 2009) were analyzed, and those cases with FS diagnosis were selected. The final diagnoses based on subsequent permanent paraffin block fixation were grouped as papillary carcinoma, follicular adenoma, multinodular goiter/ hyperplasia, Hashimoto's thyroiditis, anaplastic carcinoma, and lymphoproliferative disorders. These were compared with the correspondent FS diagnosis and classified as concordant, concordant but deferred for permanent, or discordant. The sensitivity was calculated, and the causes for discordance were tabulated.

Results: A total of 179 consecutive cases of thyroid surgery with FS diagnosis were identified and analyzed. The distribution of final diagnoses was: papillary carcinoma, (n=36); follicular adenoma, (n= 19); multinodular goiter, (n=64); Hashimoto's thyroiditis, (n=19); Hurthle cell adenoma, (n=6); lymphoproliferative diseases, (n=5); anaplastic carcinoma,(n= 4). Overall, the sensitivity of frozen section diagnosis for thyroid pathology in our institution was 75%. The sensitivity of frozen section for papillary carcinoma was 50%. Papillary carcinoma was an incidental finding in 8 cases (24.2%), found after additional sampling for permanent sections. The sensitivity for the diagnosis of follicular adenoma was 89%, for multinodular goiter 95%, for Hurthle cell adenoma and anaplastic carcinoma 100%, and for lymphoproliferative disorders was 80%. For Hashimoto's thyroiditis the sensitivity was 26%. Six of these discordant Hashimoto cases had diagnosis of papillary carcinoma, whereas three were diagnosed as follicular adenoma.

Conclusions: The sensitivity of frozen section in our institution is comparable with that of others reported in the literature, and it remains a useful resource for decisions regarding the extent of the surgical procedure. Factors such as suboptimal sampling, freezing artifact, and the experience of the pathologist interpreting the slides play a role in the concordance with the permanent H&E sections. Awareness of these factors and a methodical approach to handling and sampling the specimen is recommended to limit potential pitfalls.

1228 Detection of $BRAF^{V600E}$ Mutation in Papillary Thyroid Carcinoma by Highly Sensitive Single Strand Conformation Polymorphism Analysis

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Background: Papillary thyroid carcinoma carries a high prevalence of $BRAF^{V600E}$ activating mutation, which may serve as a diagnostic molecular marker. When in doubt, particularly with fine needle aspiration (FNA) cytological specimens, detection of the mutation essentially confirms the presence of papillary thyroid carcinoma. However, FNA specimens frequently contain fewer tumor cells, requiring highly sensitive methods of $BRAF^{V600E}$ mutation detection.

Design: DNA samples were extracted from formalin-fixed paraffin embedded tissue samples of 39 classical papillary thyroid carcinomas. BRAF gene was amplified by PCR using primers flanking the exon 15. PCR products were then resolved by single strand conformation polymorphism (SSCP) gel electrophoresis and by direct sequencing of PCR products. Analytic sensitivity of SSCP detection of $BRAF^{V600E}$ was performed by serial dilution experiments using mutation positive samples admixed with wild-type tonsil DNA.

Results: 37 of 39 classical papillary thyroid carcinomas provided amplifiable DNA, leading to informative SSCP and sequencing results. $BRAF^{V600E}$ was detected in 28 of 37 cases (76%) by SSCP. Direct sequencing failed to identify four $BRAF^{V600E}$ positive cases detectable by SSCP. Dilution studies demonstrated that SSCP had an analytical sensitivity of 5% tumor cells for a definite identification of $BRAF^{V600E}$.

Conclusions: $BRAF^{V600E}$ was seen in 76% of papillary thyroid carcinomas in our study cohort. SSCP is superior to direct sequencing of PCR product for the detection of the $BRAF^{V600E}$ with a high analytic sensitivity, making it an ideal method for molecular confirmation of papillary thyroid carcinoma, particularly when a specimen contains a few tumor cells.

1229 Ewing Family of Tumors of the Sinonasal Tract

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Background: Ewing family of tumors (EFT) are malignant neoplasms typically arising in bone and soft tissue of children and young adults. Occasional cases have been described in the sinonasal tract. Most of these have not had molecular confirmation. EFT is known for its morphologic and immunohistochemical variability making this diagnosis challenging in this location. A large series of EFT in the sinonasal tract is lacking.

Design: Cases of EFT were retrieved from the archives of the authors institutions and reviewed. PAS and PASD were available as well in most cases. Immunohistochemical stains for keratins, CD99, S100, synaptophysin, p63, desmin and myogenin were performed. One case had previously been tested with RT-PCR for EWS-FLI1. Fluorescence in-situ hybridization (FISH) was performed in cases with available tissue.

Results: Twelve cases were identified including 6 males and 6 females ranging from 7-69 yrs (mean 30.7 yrs). Tumors were left sided (4), right sided (4), bilateral (1) and unspecified (3). They presented with nasal obstruction and occured in one or more sinuses (6), nasal cavity (3) or both (3). Sinuses involved were maxillary (5), ethmoid (4) and sphenoid (2). Five cases involved orbit or dura. Nine cases invaded bone. EFT's were composed of nests, cords and sheets of small round cells with variable cytoplasmic clearing and small nucleoli. Minimal mitotic activity was present. Four cases had necrosis. Many cases showed nesting with intervening fibrovascular stroma mimicking a carcinoma or esthesioneuroblastoma. Three cases had pericellular pink material highlighted with PAS. The tumors were positive for CD99 (12/12) and focally for keratins (4/11), synaptophysin (5/9) and S100 (2/9). All cases were negative for desmin, myogenin and p63. One case showed an EWS-FLI1 fusion by RT-PCR and an additional 6 cases were positive for EWS rearrangement by FISH. No material was available in the remaining cases. Follow up in 6 cases ranging from 1-26 months showed 1 patient dead of local and distant disease (breast), 1 patient dead with local disease, 1 patient alive with lung mets and the remainder disease free.

Conclusions: EFT is an aggressive disease of the sinonasal tract with a great degree of histological diversity. It shows a greater tendancy for nesting than its soft tissue counterpart.

1230 Gulp1, an Adaptor Protein Required for Clearance of Apoptotic Cells, Is Frequently Inactivated by Promoter Methylation in HNSCC

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Background: Despite multimodality treatment, the overall survival for patients with HNSCC remains dismal. Therefore, it is of ulmost importance to identify new molecular targets that can be used for early tumor development, prediction of behavior and development of personalized, more effective antitumor therapies. Using gene expression microarray in combination of DNA demethylation by 5-azacytidine, we tentatively identified some novel genes that are epigenetically regulated in HNSCC. One such candidate gene is Gulp1, an evolutionarily conserved adaptor protein required for efficient engulfment of apoptotic cells by phagocytes. In this study, we further analyze epigenetic regulation of Gulp1 expression in HNSCC.

Design: The promoter hypermethylation in the Gulp1 gene was identified by PCR-based restriction fragment length polymorphism (RFLP) using primers specific for the Gulp1 promoter CpG island, methylation specific PCR (MSP) and further verified by direct bisulfite DNA sequencing in 11 HNSCC lines and one normal keratinocyte cell line. HNSCC and normal keratinocyte lines treated with or without DNA demethylating agents (gossypol and 5-azacytidine) were analyzed for Gulp1 mRNA expression by real-time RT-PCR. Gulp1 promoter methylation was also analysed in 154 archival HNSCC cases using MSP.

Results: 6 of 11 (54%) HNSCC lines showed promoter hypermethylation for the Gulp1 gene by both RFLP and MSP methods. Gulp1 promoter methylation was significantly reduced by the treatment of a DNA demethylating agent (5-azacytidine or gossypol) as detected by both Direct bisulfite DNA sequencing and MSP. Correspondingly, the expression of Gulp1 was significantly increased following the treatment with DNA demethylating agent. By MSP, 76 of 154 (49%) archival primary HNSCC displayed promoter hypermethylation of the Gulp1 gene.

Conclusions: Gulp1 may represent a newly identified candidate tumor suppressor gene that show frequent epigenetic inactivation in HNSCC.

1231 Carcinoma In Situ and p16 Status of Head and Neck Squamous Cell Carcinomas

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Background: The etiologic relationship of Human Papillomavirus (HPV) and squamous cell carcinoma (SCC) in the upper aerodigestive tract (UADT) is well-established. The majority of HPV-associated SCCs are non-keratinizing and located in the oropharynx (base of tongue and palatine tonsil), and are known to have a better prognosis than non-HPV-associated SCCs. Here, we report the presence of either immature or maturing / keratinizing carcinoma in situ (CIS) in a case-controlled series of p16-immunoreactive and nonreactive UADT SCCs.

Design: Consecutive biopsies of SCC in the UADT with available p16 immunohistochemistry that were diagnosed between 2007-2009 were reviewed. All cases were examined for the presence of immature CIS (non-keratinizing and similar in appearance to cervical CIS) or maturing / keratinizing CIS. Immunohistochemistry had been performed using an antibody to $p16^{INK4}$ (CINtec, MTM Laboratories), and cases interpreted as positive demonstrated nuclear and cytoplasmic staining in greater than 60% of tumor cells.

Results: 132 biopsy cases of SCC were identified (104 men and 28 women; average age 57.9 years). 57 cases were p16-immunoreactive. These were distributed as follows: hypopharynx (1), larynx (12), nasal cavity (1), nasopharynx (1), oral cavity (4), oropharynx (37), and sinonasal (1). Of the p16-immunoreactive SCCs, 35 cases were associated with immature CIS and 16 tumors were associated with maturing / keratinizing CIS. 75 biopsy cases were p16-nonreactive. These were distributed as follows: hypopharynx (5), larynx (31), oral cavity (22), oropharynx (15), and sinonasal (2). Of the p16-nonreative SCCs, 10 cases were associated with immature CIS and 46 tumors were associated with maturing / keratinizing CIS. Immature CIS was more frequently seen in the p16-immunoreactive SCCs ($p<0.01$).

Conclusions: This biopsy series of SCCs in the UADT shows the expected location distribution of p16-immunoreactive UADT SCCs, which occur more frequently in the

oropharynx. Furthermore, immature CIS is observed significantly more frequently in association with p16-immunoreactive tumors. This feature may be used to help triage cases of UADT SCC toward p16 and HPV testing.

1232 Sinonasal Malignant Melanomas (SNMM): A Clinicopathologic Review of 17 Cases

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Background: Primary SNMM is rare compromising <4% of sinonasal tumors. Given its rarity, the literature is limited to case reports and a few clinical series. The aim of this study was to review the clinicopathological features of a contemporary series of primary SNMM.

Design: A retrospective search was performed to identify cases of primary SNMM at two university medical centers. Clinical, radiographic, and follow up information was obtained from medical records. Histological sections were reviewed by two investigators to determine the dominant histologic pattern, mitotic rate, and the presence/absence of pigmentation, necrosis, ulceration, vascular invasion, and host-associated lymphocytic response. The findings were correlated with follow up information.

Results: Between June 1993 and January 2008, 17 cases were identified including 1 melanoma in situ. There were 10 men and 7 women, 52 to 88 years of age (mean, 70). The tumor was limited to the nasal cavity in 11 patients (65%), and 2 patients each showed involvement of the maxillary sinuses, ethmoid sinuses, and skull base. The invasive melanomas displayed a spindle, plasmacytoid, epitheloid, or a small blue round cell morphology in 6 (38%), 5 (31%), 4 (25%) and 1 (6%) cases, respectively. Eight (50%) cases were non-pigmented; 15 (94%) showed at least focal necrosis and 11 (69%) each showed ulceration, vascular invasion or host lymphocytic response. Mitotic rate ranged from 2 to 60/10 HPFs (mean, 20; median, 14). One patient died in the immediate postoperative period. After a median follow up of 17 mo (range, 4 to 117 mo), all patients with invasive melanoma developed recurrences and/or metastasis. Local recurrences developed in 13 patients after a median of 10 mo (range, 1 to 105 mo) and distant metastasis developed in 10 patients after a median of 12 mo (range, 1 to 36 mo). Twelve patients died with disease after a median of 16 mo (range, 4 to 117 mo). None of the evaluated clinical or histological findings were associated with a particular outcome.

Conclusions: SNMM are morphologically quite variable, often non-pigmented and are associated with an aggressive course whereby almost all patients develop recurrences and/or metastases often within a short period. Although the absence of an invasive component might be associated with a better outcome, clinical and pathological features that may predict outcome, and/or could influence therapy, remain to be determined.

1233 Correlation of Microvascular Density (MVD) with Pathological Features and Outcomes of Mucosal Malignant Melanoma (MMM) of the Head and Neck

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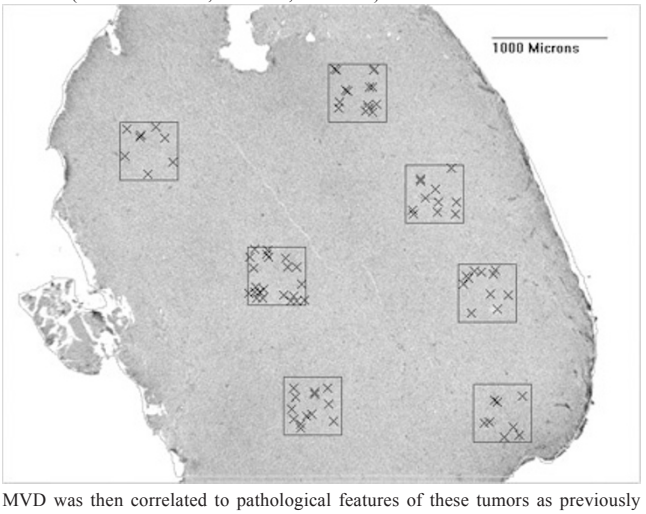
Background: Unlike its cutaneous counterparts, prognostic markers for primary MMM have not been identified. It has been recently demonstrated that MVD in cutaneous MM has a significant negative correlation with survival; however, this has not been well-studied in MMM. This study explores the potential association between MVD, various histological parameters, and the outcome of a series of MMM of the head and neck.

Design: A retrospective search was performed to identify cases of primary MMM of the head and neck at two large university medical centers. All cases were immunostained with CD31 antibody (Dako) using an automated immunostainer (Benchmark XT, Ventana). The MVD was calculated for each case by using the BioquantR Image Analysis software (R&M Biometrics, Nashville, Tennessee).



MVD was then correlated to pathological features of these tumors as previously determined by a detailed clinical and pathological review as well as to outcomes.

Results: Nineteen cases were evaluated, including 16 arising in the sinonasal tract and 3 arising in the oral cavity. The 1 year, 2 year, 3 year, 4 year, and 5 year survival rates were 75%, 57%, 61%, 46%, and 46% respectively. The MVD of the tumors was variable, ranging from 25.7 to 732 vessels/mm^2 (mean, 142.8 vessels/mm^2; median, 84.7 vessels/mm^2). There was no significant correlation between the MVD and different clinicopathological features seen within the tumors, including histologic pattern, mitotic rate, and presence/absence of pigmentation, necrosis, ulceration, or host-associated lymphocytic response. There was also no correlation between the MVD and the lengths of relapse free survival and overall survival.

Conclusions: In contrast to cutaneous melanoma, results of this study suggest that MVD does not correlate with outcome in head and neck MMM. Moreover, there was no association between MVD and particular clinical or pathological features in these tumors. Further larger studies are needed because valid predictive and prognostic markers in head and neck MMM are yet to be determined.

1234 WT-1 Expression in Salivary Gland Pleomorphic Adenomas: A Reliable Marker of Neoplastic Myoepithelium

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Background: Pleomorphic adenoma is a benign salivary gland neoplasm with a diverse morphology. This is thought to be a function of the neoplastic myoepithelial cell which shows histological and immunophenotypical variability. Wilms' tumor 1 gene (WT-1) protein, involved in bi-directional mesenchymal-epithelial transition, has been detected by reverse transcription polymerase chain reaction in salivary gland tumors showing myoepithelial-epithelial differentiation. The aim of this study was to investigate the immunoreactivity of WT-1 in pleomorphic adenomas and compare the pattern of staining with p63 and calponin, two reliable markers of myoepithelial cells.

Design: Thirty one cases of pleomorphic adenoma were selected, 28 of which included normal salivary gland tissue. The myoepithelium was classified as either myoepithelial-like (juxtatubular and spindled), modified myoepithelium (myxoid, chondroid and hyaline) and transformed myoepithelium (solid epithelioid, squamous and basaloid-cribriform). Immunohistochemistry for WT-1, p63 and calponin was assessed semi-quantatively in each myoepithelial component as well as non neoplastic myoepithelial cells and inner tubular epithelial cells.

Results: There was no immunostaining of the tubular epithelial cells by any of the markers. In contrast to p63 and calponin, WT-1 did not react with normal myoepithelial cells. Cytoplasmic WT-1 staining was present in all pleomorphic adenomas and in 30 cases (97%) more than 50% of neoplastic myoepithelial cells were highlighted. p63 and calponin stained the myoepithelium in 30 tumours. In comparison, however, 50% of the cells were positive in 22 (71%) and 12 (39%) cases respectively. Staining with WT-1 showed less variability across the spectrum of myoepithelial differentiation. The difference was most marked in the transformed myoepithelium where the solid epithelioid and basaloid/cribriform growth patterns showed strong and uniform expression with WT-1.

Conclusions: WT-1 is a sensitive marker of the neoplastic myoepithelial cell in pleomorphic adenomas. The role of this protein in influencing the mesenchymal-epithelial state of cells suggests WT-1 and the myoepithelial cell has an important role in the histogenesis of pleomorphic adenomas. The staining of WT-1 in other salivary gland tumours and its potential use in diagnostic surgical pathology requires further study.

1235 Intraoperative PTH Measurement Allows Comfirmation of Double Parathyroid Adenoma as a Cause of Primary Hyperparathyroidism

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Background: Two synchronous parathyroid adenomas (double adenoma) is a rare cause of primary hyperparathyroidism, with some questioning its existence. Routine intraoperative PTH (IOPTH) measurement as an adjunct to minimally invasive parathyroidectomy now allows identification of cases in which removal of 2 glands results in cure.

Design: A database of medical, clinical, and anatomic pathology information of 1000 consecutive surgeries for hyperparathyroidism performed between 1998 and 2007 was retrospectively reviewed. All cases in which 2 glands were removed were subjected to histologic review by an endocrine pathologist in a blinded fashion. Morphologic, histologic, and cytologic characteristics were recorded. Follow up was performed via phone survey and medical record review.

Results: Twenty seven cases of double adenoma were identified from 845 cases of primary HPT (3.2%). The average age was 59 years old, with 20 women, and 7 men. The most common configuration was bilateral upper gland involvement (13 of 27, 48%). The median weight of the involved glands was 381mg (nl 35-60mg). 31 (61%) were predominantly chief cell type, 6 (12%) oncocytic cell type, 1 (2%) clear cell type, and 13 (25%) mixed. All were hypercellular, 65% had a rim of normocelluar tissue. Oil-Red-O Sudan staining on frozen section showed decreased intracellular lipid in 53%, and completely absent lipid in an additional 28% (19% had normal amounts of intracellular lipid). IOPTH measurement of serum intact PTH was performed in all cases. The IOPTH dropped a median of 39% after the first gland was removed (from 16.3 to 11.4 pmol/ml), and 83% after the second gland was removed (to 2.3 pmol/ml). 11% of cases fell into the normal range (<6.5pmol/ml) after the first gland was removed. Of 18 patients available for long term follow up (median follow up 66 months, range 16-84), 1 patient had persistent hypercalcemia due to incomplete surgery, which was cured by later excision of the second adenoma. There were no recurrences.

Conclusions: Double parathyroid adenoma exists, although less common than previously reported. It usually involves both upper glands. It can be accurately identified with routine IOPTH measurements, and can be cured surgically without fear of recurrence. Pathologic review, including staining for intracellular fat, and comparison to biopsies of normal glands, is important in confirming the diagnosis.

1236 Clinical Significance of p16 Positive but HPV Negative Oropharyngeal Squamous Cell Carcinoma

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Background: It is now generally accepted that human papilloma virus (HPV) related squamous cell carcinomas (SCC) of the oropharynx have more favorable clinical outcome than HPV unrelated tumors. It is therefore important to accurately identify tumors that are HPV driven. For this purpose, most pathologists use a combination of both p16 immunohistochemistry (IHC) and HPV in situ hybridization (ISH). A significant minority of tumors are p16 positive but ISH HPV negative, the significance of which is unclear. The purpose of this study was to compare the clinical outcome in patients with p16 positive, HPV negative tumors to those with tumors that are positive for both p16 and HPV.

Design: 234 oropharyngeal SCC with clinical follow-up were identified from clinician databases at Washington University from 1997 to 2008. These were tested by IHC for p16 and by ISH for high-risk HPV. For p16 positive, HPV ISH negative cases, PCR was performed for high risk HPV that might not have been detected by ISH. Statistical analysis was done by log-rank tests for the equality of survivor functions and Cox Proportional Hazards regression analysis.

Results: Of the 234 cases, 187 (80%) were positive for p16. Of these, 139 (74%) were positive for HPV by ISH. Of the remaining 48 cases, 45 had material for PCR. 13 were positive for high risk HPV, leaving 32 p16 positive but HPV ISH **and** PCR negative SCCs. Among the p16 positive tumors, no significant differences in overall, disease specific, or disease free survival (p=0.38, 0.33, and 0.39) were observed between those that were HPV ISH positive and those that were ISH negative. Similarly, no differences were observed in overall, disease specific, or disease free survival (p=0.13, 0.12, and 0.13) between those that were HPV ISH positive and those that were ISH **and** PCR negative. However, p16 positive, HPV negative SCC had significantly better overall, disease specific, and disease free survival (p=0.0002, 0.0058, and 0.0125) than p16 and HPV negative SCC.

Conclusions: p16 positive, HPV negative oropharyngeal SCC has survival rates that are not significantly different from p16 positive, HPV positive tumors, but which are significantly better than p16 negative, HPV negative tumors. This data argues that HPV-specific testing adds little useful information in addition to p16 immunohistochemistry.

1237 Laryngeal Large Cell Neuroendocrine Carcinoma – A Defining Multi-Institutional Study

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Background: The WHO classifies laryngeal neuroendocrine carcinomas as typical carcinoid, atypical carcinoid (AC) or small cell carcinoma. The 5 and 10 year survival rates for laryngeal AC are 50% and 34%, respectively, and for small cell carcinoma are far worse. We hypothesized that a distinct laryngeal large cell neuroendocrine carcinoma (LCNEC) can be identified by the WHO pulmonary classification scheme that is associated with a poorer outcome than AC. Our objective was to identify new cases as well as adequately documented reported cases of LCNEC, and to correlate findings with demographic and survival data.

Design: We searched the pathology files of Washington University for the term "neuroendocrine" and anatomic site "larynx". The following histologic criteria were requisite: neuroendocrine morphology, moderate to abundant cytoplasm, high mitotic rate (>10/2mm2), and positive neuroendocrine marker immunohistochemistry (IHC). Small cell carcinoma was excluded. A literature search was also performed for cases of laryngeal neuroendocrine carcinoma, and cases which could be clearly classified as LCNEC were captured. Cases were also solicited from the larger head and neck pathology community.

Results: Six new cases of LCNEC were identified (from 1984 to 2009), along with four cases previously reported in the literature. There were 8 men and two women and 86% were smokers. Eight of 10 patients (80%) presented with lymph node metastases. Nine tumors were supraglottic and one glottic. Histologically, LCNEC was composed of large, pleomorphic tumor cells within the submucosa. Mitotic activity ranged from 48 to 158 per 10 high power fields (average 90). Necrosis ranged from spotty to extensive. Six of 8(75%) developed distant metastases, all to lung or liver. Six of 9 (66%) died of disease, all within three years. Three-year overall survival was 25%. By comparison, the 3 year survival for AC is around 50% (Wenig, Woodruff).

Conclusions: Laryngeal LCNEC is a rare entity and distinct from AC. The sole criterion to distinguish it from AC is mitotic rate, but other histologic features are usually different as well. We recommend that laryngeal tumors fulfilling these criteria not be classified as variants of AC, but rather as a form of high grade (or poorly-differentiated) neuroendocrine carcinoma, as the prognosis is poor.

1238 Lymphocytic Host Response – An Adaptive T Cell Response at Tumor Interface & Relationship with HPV

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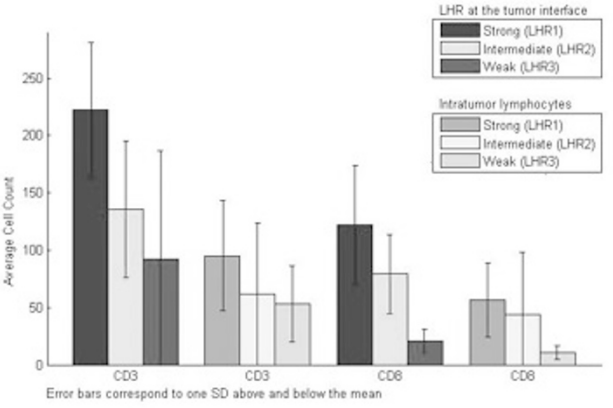
Background: Lymphocytic host response (LHR) in the Risk Model is quantified as density of inflammatory cells at the tumor interface. It is classified as **strong (LHR1), moderate (LHR2) or limited (LHR3)** and associates with the risk of disease progression. HPV16 is associated with a subset of HNSCC, and HPV+HNSCC may respond better to chemoradiotherapy with longer survival, although the mechanism is still unclear. We test two hypotheses: 1) Strong LHR corresponds to an adaptive cytotoxic T cell response, 2) transcriptionally active HPV+ HNSCC are associated with strong LHR.

Design: We compared oral resections with strong **(15),** intermediate **(19)** and weak **(4)** LHR. CD3, CD4, CD8, & CD20 cells were quantified at 40X with a grid; counting 10 densest fields at tumor interface and within tumors. Mean counts/tumor were analyzed by the 2-sided T-test. Frozen SCC from a larger set of 63 SCC were tested for active HPV16 E6/E7 by RT-PCR and corresponding LHR was assessed.

Results: Tumors with ***strong LHR 1*** have significantly increased *CD3* & *CD8* counts ***at the tumor interface*** compared to ***intermediate LHR 2*** ($p<0.001$, $p=0.009$, respectively). There were no differences with CD20 (interface & intratumor) or CD4 (interface) in LHR1 & LHR2 SCC. CD3/CD4/CD8 cell counts ***within*** tumors, across LHR groups were not significantly different, but trends were suggested. Figure 1 shows mean cell counts (CD3/CD8) at the tumor interface and within tumors, for strong, intermediate, and weak LHR.



A trend was seen with active HPV and LHR1 (score test for trend of odds p = 0.081). Tumors with transcriptionally active HPV infection were likely to have strong LHR1 when adjusted for tumor site and stage (Mantel Haenszel odds ratio 1.65, 95% CI 0.5, 5.9), albeit not signficantly.

Conclusions: Immune response at the tumor interface correlates with an adaptive T cell response, and strong LHR correlates with increased cytotoxic T cells at the interface. The association between active HPV16 and LHR1 supports increased immune surveillance as one mechanism of enhanced survival of patients with HPV+HNSCC. Our next goal is to quantify activated vs. post-activated CD8 and CD4 cells by immunoflourescence.

1239 Adenosquamous Carcinoma of the Head and Neck: Relationship to HPV

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Background: Adenosquamous carcinoma (ADSC) of the head and neck is believed to be an aggressive variant of squamous cell carcinoma (SCC) with poor patient outcome. We and others have shown that some variants of SCC of the upper aerodigestive tract are HPV related and have better prognosis than those that are unrelated. While it has been demonstrated that some adenosquamous carcinomas of the uterine cervix are HPV driven, the relationship of HPV to ADSC of the head and neck has so far not been investigated. The purpose of this study is to evaluate HPV relationship in a group of ADSC of the head and neck, using in situ hybridization (ISH) for high risk HPV and immunohistochemical stains for p16 and p53.

Design: We searched Washington University Pathology Department files for the term "adenosquamous and head and neck subsites" and found cases from 1998 to 2009. The slides were reviewed by 2 of the study pathologists. The requisite histologic criteria were the presence of squamous carcinoma combined with distinct gland formation and/ or intracellular mucin. Mucin staining was not required. In-situ hybridization for high-risk HPV and immunohistochemistry for p16 and p53 were performed.

Results: Of the 18 cases identified, 8 were from the larynx and hypopharynx, 4 from the oral cavity, 3 from the oropharynx, and 3 from the nasal cavity. The average age of the patients was 59.7 years and 16 patients were male. ISH for HPV and Immunohistochemical results are shown in the table. The majority of cases 14/18 (78%) were p53 reactive. Only three cases (16%) were positive for both HPV and p16, one from the nasal cavity* and two from the oropharynx.**

	HPV+	HPV-	p16+	p16-	p53+	p53-
Larynx/hypopharynx (N=8)	2	6	2	6	5	3
Oral (N=4)	0	4	1	3	3	1
Nasal (N=3)	1*	2	1*	2	3	0
Oropharynx**(N=2)	2**	1	2**	1	3	0

Conclusions: Adenosquamous carcinomas of the head and neck are a heterogeneous group of tumors. Only a small minority of cases are HPV related as documented by ISH and P16 immunoreactivity. HPV related ADSC are located in the oropharynx and nasal cavity, sites with known high prevalence of HPV related carcinomas. This supports the findings in the uterine cervix that HPV- related tumors may adopt the morphology of SCC variants, such as ADSC, basaloid, or papillary. The clinical significance of these findings in terms of patient outcome is the subject of further investigation.

1240 ProEx C Is Comparable to p16 and HPV ISH for the Detection of High-Risk HPV in Head and Neck Squamous Cell Carcinomas

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Background: ProEx C is a surrogate marker for HPV that targets cell cycle proteins, MCM2 and TOP2A, and has been shown to perform similarly to p16 as an aid in the diagnosis of cervical dysplasia. However, ProEx C expression has not been well-

characterized in head and neck squamous cell carcinomas (HNSCC). The purpose of this study is to determine if ProEx C performs similarly to p16 in HNSCC and to evaluate the Ventana HPV16 in situ hybridization (ISH) in comparison with HPV PCR.
Design: The study set consisted of 84 cases of HNSCC with HPV PCR results and survival data. Two cores from each case were included in a tissue microarray (TMA) which was stained with ProEx C (BD, Franklin Lakes, NJ), p16 (mtm, Westborough, MA), and HPV16 ISH (Ventana, Tucson, AZ). ProEx C was also performed on whole sections of each case. ProExC nuclear staining was scored as negative, 1+ weak (<1/3), 2+ moderate (1/3-2/3) or 3+ strong (>66%). p16 staining was scored as negative, 2+ focal strong (5-80%) or 3+ diffuse strong (>80%). HPV ISH was scored as positive (punctate or diffuse nuclear reactivity) or negative.
Results:

	ProEx C (3+) Whole	ProEx C (2-3+) Whole	ProEx C (3+) TMA	ProEx C (2-3+) TMA	p16 (3+) TMA	p16 (2-3+) TMA	HPV ISH TMA
Sensitivity	85.7% (30/35)	94.4% (34/36)	52.8% (19/36)	77.8% (28/36)	66.7% (24/36)	83.3% (30/36)	61.1% (22/36)
Specificity	72.3% (35/48)	18.8% (9/48)	77.1% (37/48)	20.8% (10/48)	95.8% (46/48)	83.3% (40/48)	93.8% (45/48)

Cases that were positive for ProEx C, p16, and/or HPV ISH showed a positive correlation with improved overall survival and increased time to nodal and distant metastasis using Kaplan Meier and Mantel-Cox log rank tests.
Conclusions: ProEx C has high sensitivity and specificity for the presence of HPV when greater than 2/3 of the tumor exhibits nuclear staining. If a lower cut-off of 1/3 is used, sensitivity is improved but with a marked decrease in specificity. Overall, ProEx C is comparable in sensitivity to p16 and HPV ISH but is less specific. In addition, ProEx C performs better on whole tissue sections than on TMA cores, suggesting that staining results on small biopsy specimens may be less reliable than on resection specimens. As with p16, ProEx C and HPV ISH have prognostic value with positive correlation with increased time to failure and improved patient survival.

1241 Tall Cell Variant of Papillary Carcinoma and Papillary Thyroid Carcinoma with Tall Cell Features Are Often Under-Diagnosed: A Retrospective Analysis of Cases Referred for Second Opinion for Clinical Management

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Background: In 1976, Hawk and Hazard described a variant of papillary thyroid carcinoma (PTC) characterized by cells with classic PTC nuclei and eosinophilic cytoplasm that were twice as tall as they were wide. This "tall cell" variant (TCV) of PTC was shown to behave in a more aggressive fashion than classic PTC. Several studies have shown TCV to be an aggressive form of PTC which has a 25% mortality rate at 10 years. Since the treatment of TCV and PTC with tall cell features (TCF) may differ from the treatment of classic PTC, the recognition of TCs in PTC is important for treatment decisions. We observed a recent lack of recognition of TC changes in patients with PTCs referred to our institution for further clinical management and examined the potential under-diagnosis of TCV and PTC with TCF in our referral patient population.
Design: A retrospective analysis of microscopic slides referred to our institution over a 5 year period (2003-2008) forms the basis of this report. Patients diagnosed with PTC and referred to our institution for additional clinical management for primary or recurrent disease were studied. In a percentage of these patients our pathology group made a diagnosis of TCV or PTC with TCF on referred slides. The pathology reports from the referring institutions were reviewed to determine the original diagnoses rendered on the slides.
Results: A TC was defined 2-3 times tall as wide and having classic PTC nuclei. TCV was diagnosed when 50% or greater TCs were present while PTC with TCF was diagnosed when <50% of the tumor contained TCs. We analyzed 225 PTC specimens referred for second opinion for clinical care and identified 40 patients (18%) in which the diagnosis of TCV (16) or PTC with TCF (24) was rendered on our review. The diagnoses rendered at the referring institution revealed TCV or PTC with TCF in only 6/40 patients (14%). In the remaining 86% of patients, no mention of "tall cell" was present in the report. 26/40 patients subsequently developed nodal metastases and 2/40 patients developed distant metastases to lung and brain at 12 years and <1 year after initial diagnosis respectively. Neither of these two cases mentioned "tall cells" in the original surgical pathology report.
Conclusions: Despite being a recognized variant of PTC for over 30 years, TCV is often under-diagnosed. This may lead to under treatment of this potentially aggressive variant of PTC.

1242 Fungal Rhinosinusitis: A Retrospective Review of 397 Patients at a Single University Medical Center

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Background: Sinonasal fungal disease (SNFD) is a well known entity, but only in more recent times have the types of SNFD been defined. In this study, we evaluate the diagnosis of SNFD in a single university medical center.
Design: The Surgical Pathology archives were searched for all specimens with a diagnosis of SNFD. If available, microscopic slides were reviewed and using the recently described guidelines proposed by International Society of Human and Animal Mycology (ISHAM) (Laryngoscope, 2009), the specimens were divided into 2 categories, non-invasive and invasive SNFD. Non-invasive SNFD included fungus ball/saprophytic fungal infection (FB) and allergic fungal rhinosinusitis (AFRS) and invasive SNFD included acute necrotizing fungal rhinosinusitis (ANIFRS), chronic invasive fungal rhinosinusitis (CIFRS), and chronic granulomatous sinonasal fungal infection (CGFRS). Fungal culture data, if available was reviewed.
Results: 397 patients with SNFD were identified. 88% were non-invasive (194 AFRS (49% overall) and 154 FB (39% overall)) and 11% were invasive (44 with ANFRS (11% overall), 5 with CIFRS (1.3% overall), 1 with CGRS (0.25% overall). 84% of AFRS had positive fungal cultures with either *Aspergillus sp*. (35%) or Dematiaceous sp. (36%) being the most common fungi isolated. 16% of AFRS patients grew more than one fungal pathogen. 50% of fungal balls had positive cultures with *Aspergillus sp*. being most (69%) followed by dematiaceous fungi (14%). ANIFRS most commonly grew *Aspergillus sp*. (50%) followed by *Rhizopus sp.* (33%) and other fungi including *Alternaria sp.*, *Paecilomyces sp* and *Fusarium sp.* (17%). >30% of ANFRS had negative fungal cultures. Cultures from the 5 patients with CIFRS grew *A. fumigatus* (1), *Scedosporium apiospermium* (1), *Candida albicans* (1) or were negative (2). Cultures were not performed on the patient with CGFRS.

Diagnosis		# (%)	% cases overall
Non-invasive (87%)			
	AFRS	194 (55%)	49%
	FB	153 (45%)	39%
Invasive (13%)			
	ANIFRS	44 (88%)	11%
	CIFRS	5 (10%)	1.3%
	CGRS	1 (2%)	0.3%

Conclusions: In our experience, most SNFD is non-invasive and most commonly AFRS. This may be secondary to patient referral for surgery at our institution. Invasive SNFD is rare compared to non-invasive disease with ANIFRS representing >90% of invasive specimens. Culture data supports that a variety of fungal agents are responsible for SNFD, but *Aspergillus sp*. appears to be one of the most common agents in patients with non-invasive and invasive SNFD in our experience.

1243 *In Situ* Hybridization (ISH) for Species Specific Fungal rRNA in Acute Necrotizing Invasive Fungal Rhinosinusitis

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Background: Acute necrotizing invasive fungal rhinosinusitis (ANIFRS) is an angioinvasive fungal infection which almost alway occurs in immunosuppressed patients. ANIFRS is most commonly caused by Aspergillus or Zygomyces infections and rarely other pathogens. A diagnosis of ANIFRS requires rapid intervention, but cultures may be negative. Identification of particular fungal subtypes is important for patient management. ISH for rRNA may be able to identify fungal organisms ANIFRS.
Design: 25 patients (58 specimens) with ANIFRS were identified. These included culture proven cases of *Rhizopus sp* (6), *Aspergillus sp* (9), *Fusarium sp* (1), *Alternaria sp* (1) and *Paecilomyces sp* (1) and 7 patients with negative cultures. Rapid (<3 hours) ISH for fungal RNA was performed using species specific biotin-labeled oligonucleotide DNA probes targeting rRNA of *Aspergillus sp*, *Fusarium sp, Rhizopus sp* and a rRNA sequence seen in a variety of Dematiaceous fungi. Preservation of fungal rRNA was determined using a pan-fungal rRNA probe.
Results: ISH with the panfungal probe showed fungal rRNA preservation in 35/58 specimens (60%). Preserved rRNA was identified in at least one specimen from 20 patients (6/6 *Rhizopus sp*, 7/9 *Aspergillus sp,* 1/1 *Fusarium sp*, 1/1 *Alternaria sp,* 1/1 *Paecilomyces sp* and 4/7 negative). ISH confirmed specific fungal rRNA in all rRNA preserved cases with the exception of the *Paecilomyces sp* case which was negative with the Dematiaceous sp probe. Negative controls consisted of ISH with the specific probes in the other fungal infections which in all cases were negative with the exception of the *Aspergillus sp* probe which resulted in weak staining in 3 of the *Rhizopus sp* cases although signal was minimal compared to that seen with the *Rhizopus sp* probe. Only 4/7 patients with negative cultures had preserved rRNA. Of these, 2 cases of *Aspergillus sp* and 1 case of Dematiaceous sp was identified by ISH.
Conclusions: 60% of ANIFRS had preserved fungal rRNA most likely due to tissue necrosis and decalcification. ISH can identify *Aspergillus sp*, *Rhizopus sp, Fusarium sp* and some Dematiaceous sp in ANIFRS. Controls must be performed and care taken not to over interpret weak signals especially when the *Aspergillus sp* probe is used in *Rhizopus sp* cases. rRNA ISH may aid fungal species identification in specimens with negative cultures but in this study which included 7 culture negative cases, only 4 had preserved rRNA with 3 additional specific fungal infections identified.

1244 Human Papillomavirus Status by In-Situ Hybridization and Correlation with p16 Immunohistochemistry in Adenoid Cystic Carcinoma of the Head and Neck

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Background: Although chronic alcohol and tobacco use are well-known risk factors for head and neck carcinoma, 15 to 20% of patients have no exposure to these substances. Reports indicate that human papillomavirus (HPV) is associated with head and neck squamous cell carcinomas (HNSCCs), particularly those involving the oropharynx and those with a basaloid phenotype. P16 expression and HPV status are not well described in other head and neck carcinomas, including those included in the differential diagnosis with basaloid SCCs. The purpose of this study is to determine status of p16 and HPV in adenoid cystic carcinomas (ACCs) of the head and neck.
Design: Tissue microarrays were constructed from 26 archival formalin-fixed, paraffin-embedded ACCs. Each tumor was represented by 3 cores. The Ventana HR HPV III probe set was used to perform automated ISH on the microarrays. IHC was performed using an antibody to p16INK4 (CINtec, MTM Laboratories). Antibody to p16 was determined to be immunoreactive if strong nuclear and cytoplasmic staining was present in >5% of cells and nonreactive if ≤5% stained.
Results: Of the 26 ACCs, 14 were immunoreactive with antibodies to p16 (54%), but none of these cases showed HPV by ISH. No p16 non-reactive cases demonstrated HPV.

Of the 14 p16 immunoreactive tumors, 13 were cribriform, tubular, or mixed pattern (93%); only 1 of 14 was solid (7%). Six of the 12 p16 non-reactive tumors were mixed (50%); 4/12 (25%) were solid; 2 were tubular, but none were pure cribriform.
Conclusions: In contrast to the good correlation between p16 IHC and HPV ISH seen in HNSCCs, there is essentially no correlation between p16 immunoreactivity and presence of HPV in ACCs. Loss of p16 may be associated with a worse prognosis as it was more often associated with a solid growth pattern. Given the high rate of p16 immunoreactivity in ACCs, p16 cannot be used to distinguish ACC from basaloid squamous cell carcinoma. However, HPV in situ hybridization may be helpful.

1245 Extra- Versus Intratumoral Perineural Invasion in Non-Cutaneous Head and Neck Squamous Cell Carcinomas

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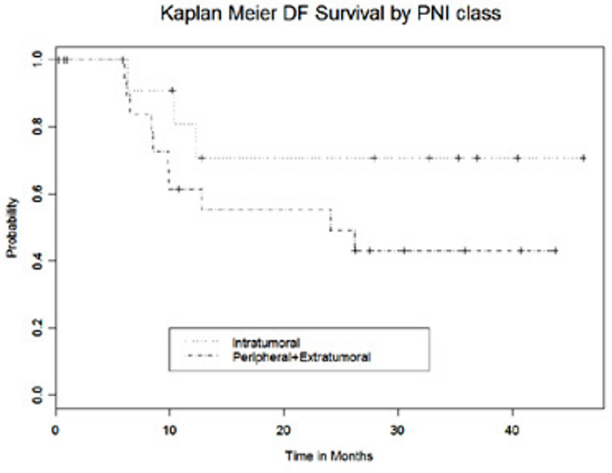
Background: Perineural invasion (PNI) in non-cutaneous head & neck squamous cell carcinoma (HNSCC) is associated with an increased risk of recurrence & decreased survival. It is currently unknown whether extratumoral (ET) PNI imparts a worse prognosis than intratumoral (IT) PNI in HNSCC.
Design: A retrospective review of 60 HNSCC cases from 1/05-5/06 was performed to identify foci of PNI. For each case, PNI density (# PNI foci/# tumor sections), size of largest nerve involved, & distance of each PNI focus to tumor edge (negative value = IT; 0 = at tumor edge; positive value = ET) were measured. PNI was categorized as IT, ET, or peripheral with the latter defined as 0 to -0.2 mm from the tumor edge. A Cox regression model was used to evaluate the effect of tumor (T) stage, nodal (N) stage, lymphovascular invasion (LVI), & PNI on disease-free (DF) survival. Kaplan-Meier survival analyis was performed to evaluate DF survival between IT, ET, & peripheral PNI groups.
Results: Of the 60 patients, 40 were male & 20 were female with a mean age of 61.5 yrs ± 12.8 yrs. There were 34 PNI cases (IT=11, P=7, & ET=16) & 26 cases without PNI. Using the Cox regression model, T-stage, N-stage, LVI, & PNI were not significantly correlated with recurrence or time to recurrence. Among PNI cases, there was a tendency of maximum extent of PNI toward smaller DF intervals (HR=1.24, p=0.15). Addition of T-stage, N-stage, & LVI did not affect this result substantially. Kaplan-Meier survival analysis showed a tendency toward a difference in DF survival between PNI class, i.e., ET+peripheral versus IT PNI groups (p=0.18). No statistically significant relationship between DF survival (p=0.43) & the size of nerve involved as well as the PNI density (p=0.32) was observed.



Conclusions: Preliminary data with a limited number of cases demonstrate a tendency of maximum extent of PNI toward diminished DF survival. Moreover, ET+peripheral PNI tended to have a shorter DF survival than IT PNI. Additional cases need to be analyzed in order to elucidate the statistical significance of this trend. Other clinical and histological parameters will be evaluated.

1246 Molecular Determination of Primary Versus Metastatic Squamous Cell Carcinoma (SCC) of the Lung in the Context of SCC of the Head and Neck (H/N) — Comparison of Methodologies

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Background: Considering similar risk factors, patients with SCC H/N are at a high risk of developing SCC within the lung. These lesions are often characterized as metastatic SCC, even though they often present as solitary masses. Given similar histologies, the distinction between metastatic and primary SCC is often difficult. Thus, molecular methods may be clinically useful in the evaluation of tumor relatedness. It has previously been shown that assessment of allelic variation at the level of microsatellite sequences can be utilized to discriminate multiple primary versus metastatic SCC of H/N and lung via a rapid, sensitive, method independent of the presence of normal tissue. The method is amenable to the analysis of DNA from paraffin-embedded specimens. The goal of this study was to refine this methodology via implementation of fluorescent-labeled primers and/or multiplexed reactions with automated detection.
Design: Genomic DNA was extracted from paraffin-embedded tumor samples from nine patients who had undergone resection of SCC of both the H/N and lung. PCR was performed to determine allelic variation, and the cases were scored for mutually exclusive allelic losses. Comparison was made between microsatellite PCR with PAGE analysis versus fluorescent product detection. The same samples were analyzed with the Ampfister Identifiler multiplex (Applied Biosystems).
Results: All three methods yielded interpretable results. Samples consistent with metastatic lung lesions were characterized by either identical allelic losses or additional allelic losses as compared to SCC H/N, while lesions consistent with metachronous primary SCC of the lung were characterized by retention of alleles that were lost in the SCC H/N.
Conclusions: The use of fluorescent-labeled primers improves throughput by streamlining the analysis of PCR products. Likewise, analysis of patient samples using multiplex PCR can yield sufficient information to determine relatedness of neoplasms in a single PCR reaction. The results of this study confirm that molecular methods can distinguish primary vs. metastatic SCC of the lung in the context of SCC H/N and that practical clinical laboratory assessment of these neoplasms is feasible. Accurate characterization of these lesions may significantly impact clinical management strategies for these patients.

1247 Ectopic Pituitary Adenoma (EPA): A Clinicopathologic Study of 40 Cases

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Background: EPA may arise in various upper aerodigestive tract sites from remnants of Rathke's pouch. In these locations, misdiagnosis with other neuroendocrine/neuroectodermal tumors (eg, paraganglioma, olfactory neuroblastoma, neuroendocrine carcinoma) or with malignant epithelial neoplasms may occur.
Design: 40 cases of EPA were identified from our files. Clinical records were available in all cases; material (blocks, slides) were variably available for immunohistochemical (IHC) staining. IHC antigenic profile included staining for cytokeratins (AE1/AE3, CAM5.2), chromogranin, synaptophysin, CD56, S100 protein, neuron specific enolase, MIB-1 (Ki67) and pituitary hormones, including adrenocorticotropic hormone, growth hormone, prolactin, follicle stimulating hormone, luteinizing hormone and thyroid stimulating hormone. A requirement for inclusion was radiologic imaging showing the sella turcica/pituitary gland to be uninvolved by tumor.
Results: The patients included 24 males and 16 females ranging in age from 22-84 yrs (median, 51 yrs). Symptoms included airway obstruction, chronic sinusitis, epistaxis, visual field defects, CSF leakage, and headaches. The primary site of occurrence included the sphenoid sinus (n=27), nasopharynx (n=5), nasal cavity (n=4) and ethmoid sinus (n=4). The tumors were submucosal with solid, organoid, and trabecular growth patterns, fibrovascular stroma composed of bland cells with round nuclei, dispersed chromatin and granular eosinophilic cytoplasm. Pleomorphism, necrosis and increased mitotic activity were not seen. IHC staining was present for neuroendocrine markers (chromogranin, synaptophysin and/or CD56) in 90% and cytokeratin in 71% of cases. Reactivity with pituitary hormones included 50% reactive for 2 or more hormones (plurihormonal PA), 39% reactive for a single hormone and 11% non-reactive (null cell). MIB-1 staining in 18 cases showed a low proliferation rate (less than 1%). Surgery is the treatment of choice and often proves curative without recurrent or progressive tumor.
Conclusions: EPA primarily arises in the sphenoid sinus with characteristic light microscopic and IHC findings. Misdiagnosis and unwarranted therapy occurs due to misinterpretation of the light microscopic and IHC correlative findings.

1248 Diagnostic Utility of IMP3 Expression in Thyroid Neoplasms: A Real-Time PCR Study

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Background: Immunohistochemical detection of insulin-like growth factor II mRNA binding protein 3 (IMP3) expression levels has recently been found to be a useful marker in differentiating follicular adenoma from follicular variant of papillary thyroid carcinoma and follicular carcinoma. IMP3 is a regulator of insulin-like growth factor 2 expression and is active in embryonic tissues during development but not in adult tissues. IMP3 appears to play a significant role in the neoplastic process of a number of tissues including thyroid follicular cells. Its potential role in assisting in the distinction between benign and malignant well differentiated thyroid tumors was explored by real-time PCR.
Design: A total of 106 thyroid neoplasms were collected from the pathology files between 1980-2008, including: follicular adenomas (FA) n=22, follicular carcinomas (FTC) n=14, papillary thyroid carcinomas (PTC) n=28, follicular variant of papillary thyroid carcinoma (FVPTC) n=11, Hurthle cell adenomas (HA) n=10, and Hurthle cell carcinomas (HCA) n=21. RNA was extracted from formalin fixed paraffin embedded tissue. Real-time PCR primers and probes were designed with a fluorescent tag. Real-time PCR was performed on a LC480; samples were tested in triplicate in a 20 microliter reaction volume with 200 ng of RNA. Phosphoglycerol kinase was used as an RNA control, and a pool of three normal thyroids were used for calibration and to calculate the fold change in expression over normal.
Results: IMP3 mRNA expression in all tumors was elevated compared to normal thyroid tissue: FA 2.8 fold increase, FTC 22.2 fold increase, FVPTC 47.4 fold increase, PTC 44.7 fold increase, HA 11.6 fold increase, and HCA 12.1 fold increase. Using a cut off of 7.4 fold the sensitivity and specificity of IMP3 for the whole group was 81% and 81% respectively. However, for the non-Hurthle tumors the sensitivity was 92% and the specificity was 95% compared to that for the Hurthle cell tumors, 52% and 50% respectively. The positive predictive value and negative predictive value of IMP3 in the overall cohort was 91% and 65%, while among the non-Hurthle cell tumors it was 98% and 84% respectively and in the Hurthle cell tumors it was 69% and 33% respectively.

Conclusions: IMP3 mRNA expression is helpful in distinguishing between benign and malignant follicular and papillary thyroid tumors. However, IMP3 is not a useful marker to distinguish benign and malignant Hurthle cell tumors.

1249 Is Intratumoral (IT) or Peritumoral (PT) Lymphangiogenesis in Low-Grade Salivary Gland Carcinomas Significant?

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Background: The prognosis of mucoepidermoid (MEC), acinic cell (ACC) and adenoid cystic carcinoma (ADCY) is dependent on tumor size, nodal metastases and grade. Nodal metastases correlate with lymphatic density (LD) in some tumors like prostate and colon carcinoma but no data on lymphangiogenesis are available in salivary gland carcinomas. The aim of this study was to assess IT or PT lymphatic densities in MEC, ADCY, ACC as compared to pleomorphic adenomas (PA) and determine any relationship between LD and nodal metastases in these tumors.

Design: All ADCY, MEC, ACC and a matching number of PA diagnosed during a 10 year period in our institute were identified and reviewed. Blocks containing normal and tumor interface were immunostained with D-2-40 (Biocare Medical) using Ventana Benchmark XT. Two reviewers quantified lymphatic density (averaged from number of lymphatics in 4 high power fields in "hot spots" identified at low power) in IT and PT tissue (within 10-mm around tumor).

Results: 73% of all malignant tumors and 100% of PA originated in the parotid. 9 cases (6 MEC, 2 ACC, 1 ADCY) had lymph node metastases (LN+) at diagnosis. The lymphatic density is significantly higher in the PT tissue compared to the tumor ($p<0.0001$) for MEC, ADCY, ACC and PA. The IT and PT lymphatic densities between different malignancies do not show any significant difference ($p>0.05$) and were not different than the same measurements in PA.

Intratumoral (IT) and Peritumoral (PT) Lymphatic density

Tumor(n=47)	Age (mean)	M:F	IT(Mean+/-SD)	PT(Mean+/-SD)	p-value
MEC(n=10)	52.5	7:3	0.40+/-0.32	3.45+/-1.28	<0.0001
ADCY(n=18)	48	8:10	0.50+/-0.61	3.83+/-1.48	<0.0001
ACC(n=9)	42	4:5	0.38+/-0.99	4.00+/-1.16	<0.0001
PA(n=10)	43	5:5	0.55+/-0.64	3.77+/-1.12	<0.0001

No difference in IT lymphatic density was seen between LN+ cases and LN- ones (0.33±0.35 vs.0.48±0.73, p=0.56). The PT lymphatic density was surprisingly significantly lower in LN+ cases than LN- ones (2.67±1.25 vs. 4.05±1.08, p=0.004).

Conclusions: All tumors showed lower IT than PT lymphatic densities but no difference in IT or PT density amongst malignant tumors or between benign and malignant tumors was seen. No correlation between LD and nodal status was found. These results suggest that lymphangiogenesis does not play a major role in aggressiveness of low grade salivary gland carcinomas.

1250 Cutaneous Adenexal Differentiation in Salivary Benign Mixed Tumors

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Background: Cutaneous adenexal differentiation (CAD) is well-recognized in benign mixed tumors occurring in cutaneous sites. The incidence of this histologic finding in salivary gland sites is not known. We sought to describe the incidence of CAD in benign mixed tumors of the palate, lip, and parotid gland.

Design: Benign mixed tumors of the palate (n=29), lip (n=13), and parotid gland (n=37) resected between 1980 and 2009 at a single academic medical institution were reviewed. All hematoxylin and eosin stained sections containing neoplasm were reviewed by all authors which included one dermatopathologist (S.O.). After confirming the diagnosis of mixed tumor, we evaluated for morphologic evidence of CAD and metaplastic stromal changes. Specifically we evaluated for the presence of infundibulocystic structures, tricholemmal keratinization, shadow cells, follicular epithelial differentiation, follicular bulb or papillary mesenchymal body-like structures, and sebaceous differentiation. Chart review was performed to obtain pertinent clinical information.

Results: Cutaneous adenexal differentiation was seen in 17% of palate and 38% of lip benign mixed tumors but in no parotid tumors (Table). The most frequent features were tricholemmal keratinization (38% of lip and 17% of palate tumors), follicular epithelial differentiation (38 and 17%), and infundibulocystic structures (23 and 14%). Sebaceous differentiation was seen in only one palate tumor. Varying amounts of stromal adipose were seen in 62, 34, and 21% of lip, palate and parotid tumors. Osseous metaplasia was seen in one tumor from each site.

Table: Summary of Clinical and Histologic Features

Site	# of cases	# with CAD	% with CAD	Mean age, yrs (range)	M:F	Mean size, cm (range)
Palate	29	5	17	45 (12-81)	16:13	2.3 (0.8-6)
Lip	13	5	38	49 (27-88)	7:6	1.4 (0.4-3)
Parotid	37	0	0	48 (21-84)	11:26	2.5 (0.6-6.5)

Conclusions: Cutaneous adenexal differentiation was identified in a significant number of benign mixed tumors of the lip (38%) and palate (17%) but in no parotid tumors. The most frequent histologic features of this were infundibulocystic structures, tricholemmal keratinization, and follicular epithelial differentiation. When such changes become prominent, it can present a diagnostic pitfall that must not be over interpreted as squamous cell carcinoma at biopsy or frozen section. Our finding of CAD in 38% of benign mixed tumors of the lip reconfirms previous reports of such differentiation in up to 44% of cutaneous benign mixed tumors.

1251 T(11;19) Translocation in Mucoepidermoid Carcinoma

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Background: Mucoepidermoid carcinoma (MEC) is the most common malignancy of salivary glands. MEC is also seen in the sinonasal and lower respiratory tracts. The prognosis of MEC largely depends on tumor stage and grade. Recently the recurrent t(11;19) translocation with a CRTC1-MAML2 chimeric gene has been described in MEC. The presence of t(11;19) in MECs has ranged from 38-81% and has been has been associated with grade I-II tumors and a favorable outcome. Nevertheless, the presence of t(11;19) in grade III MEC remains controversial. The aim of this study is to investigate the presence of the t(11;19) translocation in a large series of surgically treated MUC.

Design: Seventy-three (73) MECs were retrieved from the pathology files of the University Health Network. The tumors were resected between 1992-2007. All the slides available were reviewed and the tumors were graded using the Healey's system. RNA was extracted from representative formalin-fixed, paraffin-embedded tissue from each case and the presence of the t(11;19) translocation was investigated by reverse transcriptase polymerase chain reaction (RT-PCR).

Results: Twenty-three cases (32%) were positive for the t(11;19) translocation with 22 containing the CRTC1-MAML2 chimeric gene and 1 the CRTC3-MAML2 gene indicating a likely t(11:15) translocation. The t(11;19) translocation was found in 8/23 (35%) grade I tumors, 11/35 (31%) grade II tumors, 3/12 (25%) grade III tumors, and 1/3 (33%) of oncocytic MECs. The tumor with the CRTC3-MAML2 gene was grade I. Clinical follow-up of fusion-positive MECs ranged from 2-331 months with a mean of 52. At last follow-up 18 patients were alive with no disease, 3 were alive with widely recurrent disease (22, 23, and 331 months), and 1 had died with disease (10 months). The patient that died had a T3N2Mx lesion of the base of tongue whereas those with recurrent disease had advanced sinonasal tumors at presentation.

Conclusions: The t(11;19) translocation was more common in grade I-II MECs but was also seen in 25% of grade III tumors. MECs arising in the sinonasal tract may have an adverse outcome despite the presence of t(11;19) underscoring the importance of primary tumor site and stage in the prognosis of MEC. Given the variable detection of t(11;19) using RT-PCR, there is a need to assess its incidence and clinical significance using fluorescence in-situ hybridization. Rare MECs have a variant CRTC3-MAML2 chimeric gene.

1252 Salivary Gland Lymphadenomas: Clinicopathological and Immunohistochemical Study

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Background: Lymphadenomas (LAD) are rare major salivary gland neoplasms with prominent lymphoid stroma reminiscent of Warthin tumors. Categorized as sebaceous (SL) and non-sebaceous (NSL), their clinicopathologic profile and pathogenesis is unclear. These lesions may occasionally mimic nonkeratinizing squamous cell carcinoma or lymphoepithelial carcinomas, but to date the viral status of LAD has not been described. We report the clinicopathologic and immunohistochemical features of 16 cases.

Design: LAD were collected from various institutions. Clinical information, pathologic findings and immunohistochemical expression profiles were studied.

Results: 16 LAD were identified, 10 SL and 6 NSL, in 10 women and 6 men (F:M 1.6:1) aged 42 to 78 (median 60) years. 12 tumors arose in the parotid and 1 in the submandibular gland. 15 tumors presented as masses. All tumors were well-circumscribed, grey tan to yellow and homogeneous, measuring from 0.9 to 5.1 (median 2.2) cm. A well-formed capsule and cystic change was seen in 14/16 (88%) tumors respectively. Prominent lymphoid stroma was noticed in all, with germinal centers in 11 (69%). The epithelial component comprised of basaloid cells forming solid trabeculae, with tubules and glands in 11 tumors (69%). One SL showed oncocytic metaplasia while 1 NSL showed squamous differentiation with keratinization. Immunohistochemistry (IHC) showed that the epithelial cells were positive for p63 (10/10), CK7 (6/6) and essentially negative for calponin (6/6). Ki-67 showed no or low (<5%) proliferation in the epithelial cells (4/4). Moderate to strong expression of p16 protein was noted in epithelial cells in 2 cases. EBV, HPV and HHV-8 (KSHV) viral status were negative in 3, 1 and 1 case respectively. No recurrences were noted (follow-up 1-8 year) after surgical resection.

Conclusions: LAD are benign tumors with a slight female predilection unlike Warthin tumors. Based on limited data, LAD are not virally driven which in conjunction with the well demarcated bland appearance, may be useful in distinguishing these tumors from metastatic non-keratinizing squamous cell carcinoma or lymphoepithelial carcinoma, however, strong expression of p16 protein in two cases warrants more extensive validation of this concept.

1253 Long Term Follow-Up of Patients with Classic and Follicular Variant of Papillary Thyroid Carcinoma: A Comparative Study

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Background: The two most common variants of papillary thyroid carcinoma (PTC) are classic variant (PTC-CV) and follicular variant (PTC-FV). Both PTC-CV and PTC-FV can present as completely or partially encapsulated or un-encapsulated tumors. The encapsulated PTC is often associated with an excellent prognosis. The purpose of this study is to compare the clinicopathologic features and long-term follow-up of PTC-CV and PTC-FV with special regards to the encapsulated form.

Design: A cohort of 114 cases including 58 PTC-CV and 56 PTC-FV were selected from the computerized pathology files at the Hospital of University of Pennsylvania. Clinico-pathologic data and follow-up (serum thyroglobulin measurements, radiologic studies and/or additional tissue sampling) through present date were extracted from medical records.

Results: The median patient age at initial diagnosis was 46 years for PTC-CV with a male to female ratio of 16:42 and 45.5 years for PTC-FV, with a male to female ratio

of 13:43. Complete tumor encapsulation was seen in 40 (69%) PTC-CV and in all (100%) PTC-FV cases. A higher rate of tumor capsule invasion(CI) lymphovascular invasion(LVI), extrathyroidal extension (ETE) and lymph node metastases (LN-mets) was seen in PTC-CV as compared to PTC-FV; CI 26% vs. 18%, LVI 17% vs. 4%, ETE 19% vs. 7% and LN-mets 67% vs. 29%.Clinical, radiologic and/or pathologic follow-up was available in 36 (62%) PTC-CV and 34 (61%) PTC-FV cases. The median follow-up for PTC-CV was 10-years and PTC-FV was 9-years. Tumor recurrence was found in 11(11/36-31%) PTC-CV and 2 (2/34-6%) PTC-FV cases. Distant metastasis occurred in 3 (5%) of PTC-CV and 1(1.7%) PTC-FV case; of these two cases of PTC-CV were encapsulated tumors which showed CI, LVI and LN-mets.

Conclusions: Clinical follow-up was available in 62% of PTC-CV and 61% PTC-FV patients. In our experience, encapsulated PTC-FV is an indolent disease as compared to encapsulated PTC-CV, with only 1/34 (3%) patients with long-term follow-up showing distant metastases.

1254 IgG4-Positive Plasma Cell Infiltrates in Chronic Sialadenitis Involving Submandibular Glands

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Background: Chronic sclerosing sialadenitis (CSS) is a chronic inflammatory disorder of salivary glands which most commonly affects submandibular glands. CSS has been postulated to be an IgG4-related disease with occasional cases associated with systemic manifestations. The aim of our study was to assess the frequency of increased IgG4-positive plasma cells in chronic sialadenitis involving submandibular glands, and study the possible clinical associations with other IgG4-related diseases.

Design: We studied 81 consecutive excised submandibular glands with chronic sialadenitis and no associated head and neck malignancy. Surgery was performed from 1992-2008, and follow-up ranged from 1 day to 154 months (mean 52 months). IgG4-positive plasma cells were averaged in three high power fields (hpf) and graded as negative (<5/hpf), mild (6-10/hpf), moderate (11-30/hpf) and marked (>30/hpf). Results of IgG4 stain were correlated with clinical features (age, gender, presentation, presence of IgG4-related disease, IgG4 and autoantibody levels) and histologic findings (degree of lymphoplasmacytic infiltration, fibrosis, lymphoepithelial lesions, lymphoid follicle formation and phlebitis).

Results: 27 cases (33%; M:F=5:4) showed marked (18 cases) or moderate (9 cases) increase in IgG4-positive plasma cells and were classified as IgG4-positive. These cases showed mild to marked lymphoplasmacytic infiltrate with plasma cells comprising 10% to 80% of the infiltrate. IgG4-positive cases showed increased fibrosis (100% vs. 78%, p=0.007), lymphoepithelial lesions (59% vs. 19%, p=0.0004) and association with sialolithiasis (59% vs. 24%, p= 0.003) compared to remaining cases. There was no significant difference in lymphoid follicle formation (48% vs. 32%, p=0.15) or phlebitis (0% vs. 4%, p=0.5) between the two groups. Of the 27 IgG4-positive cases, 21 (78%) presented with mass and 1 (4%) had associated lymphadenopathy. IgG4 and ANA levels were performed only in one case each and were normal. Associated IgG4-related diseases were identified in 4 cases (15%) including 2 cases with cholangitis, 1 case with orbital pseudolymphoma and 1 case with retroperitoneal fibrosis. At last follow up the patients were alive and well (23 cases) or had died of unrelated disease (4 cases).

Conclusions: Nearly a third of cases of chronic sialadenitis affecting the submandibular glands showed increased IgG4-positive plasma cell infiltrates. IgG4-positive cases had increased incidence of fibrosis, lymphoepithelial lesions and association with stones. The majority of patients did not have other IgG4-related diseases.

1255 Immunohistochemical Expression of CD117 (C-Kit) in Mucosal Melanomas of the Head and Neck

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Background: Mucosal Melanomas of the head and neck region are rare and account for 1.3% of all cutaneous and non-cutaneous melanomas. Treatments often require adjuvant radiation and/or chemotherapy, due to difficulty to completely remove those tumors by surgery alone. Recent studies showed c-kit gene mutation in skin and mucosal melanomas, suggesting the therapeutic benefit of tyrosine kinase inhibitors, such as imatinib (Gleevac). We have applied immunohistochmistry on c-kit protein and demonstrated c-kit immunoreactivity in mucosal melanomas.

Design: Twenty seven patients with mucosal melanomas from nasal cavities and para sinuses were treated in 2 major tertiary hospitals. All cases were diagnosed as primary mucosal melanomas and were confirmed by immunostains for melanocytic markers including S100 protein, HMB-45, PNL-2, and/or Melan-A and PNL-2Melan-A. 27 cases of formalin fixed and paraffin embedded samples were retrieved from 2 hospital archives. Automated Iimmunohistochemistry was performed using a pre-diluted monoclonal c-kit antibody (Clone 9.7, Ventana Medical SysytemsSystems, Tucson, AZ), by a standard avidin-biotin complex detection using the immunostainers BenchMark XT (Ventana Medical SysytemsSystems, Tucson, AZ). Internal positive (mast cells) and negative controls were identified in every stained section.

Results: Immunohistochemical expression of c-kit was determined by evaluating the membranous staining using a semi-quantitative measurement as follows: 1+ for < 25% of tumor cells, 2+ for 25-50%, 3+ for > 50+, and 4+ for > 75% of cells displaying positive staining. We found that 24 of the 27 (88 %) tumors had distinct positive membranous staining. Among those positive cases, 5 tumors were uniformly diffusely positive (4+, > 75%), 8 were strongly positive (3+, > 50%), 7 were patchy positive (2+, > 25-50 %), and 4 showed focal and weak (1+, < 25 %) staining.

Conclusions: Our results show that immunoreactivity of CD117 (c-kit) can be detected in majority of mucosal melanomas with a distinct, reproducable membranous staining pattern. Immunohistochemical expression of c-kit may be useful to provide a potential therapeutic target using tyrosine kinase inhibitors to treat mucosal melanomas.

1256 Lymphoepithelial-Like Carcinoma of the Oropharynx: A Morphologic Variant of HPV-Related Head and Neck Carcinoma

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Background: Human papillomavirus-associated squamous cell cancer of the head and neck (HPV-HNSCC) represents an important subgroup of head and neck cancer that is characterized by distinct epidemiological, clinical and pathologic features including a relatively constant microscopic appearance. For those cancers that deviate from the morphologic prototype, an association with HPV may not be recognized and accurate tumor classification may not be achieved.

Design: We have identified 22 cases of HPV-HNSCC with well developed lymphoepithelial-like features including tumor cells with syncitial cytoplasm, vesicular nuclei and large central nucleoli dispersed in an inflammatory background as cell clusters or single cells. The pattern closely resembles Epstein Barr virus (EBV)-induced undifferentiated carcinoma of the nasopharynx. Indeed, 3 of the carcinomas presenting as lymph node metastases were originally misdiagnosed as metastatic nasopharyngeal carcinoma. These cases were analyzed for the presence of HPV and EBV using p16 immunohistochemistry and viral in-situ hybridization.

Results: Unlike nasopharyngeal carcinoma, the cases were of oropharyngeal origin, p16 positive by immunohistochemistry (22 of 22, 100%), HPV-16 positive by in-situ hybridization (19 of 22, 86%) and EBV negative by in-situ hybridization (21 of 21, 100%). Like conventional HPV-related HNSCC, the cases tended to occur in patients under 60 years of age (82%), men (71%), and non-smokers (88%).

Conclusions: For carcinomas of the head and neck that exhibit lymphoepithelial features, one cannot assume an EBV-driven process by morphology alone. HPV testing has disclosed a previously unrecognized morphologic variant of HPV-HNSCC that is microscopically indistinguishable from EBV-related nasopharyngeal carcinoma. For lymphoepithelial-like carcinomas presenting as cervical lymph node metastases, testing for HPV and EBV is mandatory.

1257 Mammary Analogue Secretory Carcinoma of Salivary Glands, Containing the *ETV6-NTRK3* Fusion Gene. Hitherto Undescribed Salivary Gland Tumor Entity

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Background: We present a series of thirteen salivary gland tumors with histomorphological and immunohistochemical features reminiscent of secretory carcinoma (SC) of the breast. This is a hitherto undescribed and distinctive salivary gland neoplasm, with features of both salivary acinic cell carcinoma (AciCC) and mammary SC.

Design: The patients comprised 8 men and 5 women, with a mean age of 48 years (range 26-59). Eleven cases occurred in the parotid gland, and one each in the minor salivary glands of buccal mucosa and palate. The mean size of the tumors was 2.3 cm (range 0.9 to 5.5 cm). Duration of symptoms was recorded in 9 cases and ranged from 2 months to 30 years. Clinical follow-up was available in 11 cases, and ranged from three to ten years. Three patients suffered local recurrences. Two patients died, one of them due to multiple local recurrences with extension to the temporal bone, and another one due to metastatic dissemination to cervical lymph nodes, pleura, pericardium and lungs.

Results: Microscopically, the tumors have a lobulated growth pattern and are composed of microcystic and glandular spaces with abundant eosinophilic homogenous or bubbly secretory material positive for PAS, mucicarmine, MUC1 and MUC4. They also show strong vimentin and S-100 protein positivity. For this tumor we propose a designation mammary analogue secretory carcinoma of salivary glands (MASC). We have demonstrated a t(12;15) (p13;q25) *ETV6-NTRK3* translocation in all but one cases of MASC. This translocation was not found in any conventional salivary AciCC, whereas *ETV6-NTRK3* gene rearrangements were proven in all three cases of mammary secretory carcinoma.

Conclusions: Our results strongly support the concept that MASC and AciCC of salivary glands are different entities and should be recorded separately in salivary gland tumor classifications.

1258 Increased Fibroblast Density and Syndecan-1 Expression in the Stroma of Lip Cancer Samples

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Background: Squamous Cell Carcinoma (SCC) of the lip is a type of oral cancer that affects the lip vermillion. Its main etiologic factor is ultraviolet sunlight, and the premalignant and malignant forms of lip SCC have shown increased prevalence in countries located near the Antarctic ozone hole, such as Chile and Australia. It has been demonstrated that the stroma surrounding several neoplasias plays a crucial role in cancer progression. Syndecan-1 is a cell-surface heparan sulfate proteoglycan which is involved in cell proliferation, adhesion and migration. During carcinogenesis, syndecan-1 expression is reduced in cancer cells, whereas its expression is increased in stromal cells, specifically in activated fibroblasts. Therefore the objectives of this study were to determine fibroblast density in the stroma of lip SCC and normal lip samples and to assess if stromal fibroblasts express syndecan-1.

Design: Serial samples of normal lip (n=12) and lip SCC (n=11) biopsies were immunostained for syndecan-1 and prolyl-4-hydroxylase (fibroblast marker) detection. Samples were digitalized and analyzed at the reticular and papillar areas of normal lip, and at the intratumoral (IT) and peritumoral (PT) stroma of lip SCC to obtain

syndecan-1 expression score and fibroblast density, and to assess co-localization of fibroblasts and syndecan-1.
Results: Both syndecan-1 expression and fibroblast density were increased in lip SCC as compared to normal lip (P<0.001, Wilcoxon). In addition, fibroblasts from normal lip samples showed no syndecan-1 expression (0/11), whereas, 8 out of 11 samples of lip SCC showed syndecan-1 expression by stromal fibroblasts (P<0.0001, Fisher test).
Conclusions: The results showed that fibroblast density was significantly increased in lip SCC as compared to normal lip. In addition, fibroblasts from normal lip did not express syndecan-1, whereas, stromal fibroblasts from lip SCC showed increased syndecan-1 expression . This suggests that stromal fibroblasts have and activated phenotype in malignant lip lesions that could contribute to lip cancer growth and invasion. Supported by CONICYT, grant FONDECYT 1090287.

1259 IgG4 Positive Plasma Cells in Sclerosing Variant of Mucoepidermoid Carcinoma: A New and Evolving Concept

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Background: IgG4-related sclerosing disease is a recently described syndrome with unique histological features characterized by intense lymphoplasmacytic infiltrates with increased IgG4+ plasma cells, & dense stromal sclerosis. The disease spectrum frequently includes benign inflammatory diseases, such as sclerosing pancreatitis, cholangitis & chronic sclerosing sialadenitis (CSS). Mucoepidermoid carcinoma (MEC) is the most common primary malignancy in the salivary gland. The rare sclerosing variant of MEC is characterized by dense stromal sclerosis & lymphoplasmacytic infiltrates. Our goal was to further characterize lymphoplasmacytic infiltrates with respect to IgG4 expression.
Design: Six sclerosing MECs from our pathology service over the past 20 years were selected. In addition, 11 regular MEC with lymphoplasmacytic infiltrates, 4 CSS, & 12 cases of non-sclerosing chronic sialadenitis (CS) were evaluated. Formalin fixed paraffin embedded sections were stained for IgG4 & IgG by IHC. Images were captured under 400X magnification that correspond to a field area of 0.0675 mm^2. The number of IgG & IgG4 positive plasma cells was quantified in the same field from five different areas, & the IgG4+ to IgG+ ratio was calculated.
Results: The 6 sclerosing MECs were characterized by prominent sclerosis & dense lymphoid predominantly peritumoral infiltrates rich in plasma cells. None of sclerosing MEC patients had IgG4-related sclerosing disease. The absolute number of IgG4+ plasma cells was significantly increased in sclerosing MEC as compared to regular type (75 vs 20 per image field, P<0.05). Furthermore, the ratio of IgG4+/IgG+ plasma cells was markedly elevated in sclerosing MEC as compared to the regular type (46.5 vs 17, P<0.005). In CSS, absolute numbers of IgG+ plasma cells & IgG4+/IgG+ ratio were significantly increased as compared to CS (P<0.005).

Results

	Sclerosing MEC n=6 Mean (range)	MEC n=11	CSS n=4	CS n=12
IgG4	75 (18-190)	20 (3-42)	52 (7-108)	2 (0-11)
IgG	172 (54-279)	108 (23-212)	88 (37-152)	63 (3-288)
IgG4/IgG	46.5 (22-75)	17 (5-33)	54 (30-79)	4.5 (0-23)

Conclusions: This study is the first to demonstrate increased IgG4 plasma cells in sclerosing MEC. This increase in malignant sclerotic disease is similar to that in inflammatory autoimmune conditions such as SCC. Association of the elevated IgG4+ plasma cells with increased fibrosis in sclerosing variant of MEC suggests a role of IgG4+ plasma cells in fibrogenesis and may be a new concept related to sclerosis in cancer.

1260 Cyclin B1 and E2F-1 Expression Correlates with P16 in Head and Neck Squamous Cell Carcinomas

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Background: Cyclin B1 is a key molecule involved in the transition from G2 to M phase of the cell cycle and interacts with both pRB and p53. E2F-1 is a nuclear transcription factor that binds with pRB during the regulation of several genes needed for cell cycle entry and DNA synthesis. The expression of these cell cycle regulators in head and neck squamous cell carcinomas has not been previously reported.
Design: 64 cases of head and neck squamous cell carcinoma were retrieved for immunohistochemical analysis. The cases encompassed 20 laryngeal carcinomas, 21 tonsillar carcinomas and 21 tongue carcinomas. Immunohistochemical analysis was performed using antibodies directed against p16, E2F-1 and cyclin B1. Positive staining was quantitatively and qualitatively recorded. The fisher exact test and paired T-test were used to determine significance.
Results: p16 positive staining was seen in 2/20 laryngeal carcinomas, 13/21 tonsillar carcinomas, and 8/21 tongue carcinomas. Cyclin B1 staining and E2F-1 staining was increased in all p16 positive carcinomas compared with all p16 negative carcinomas (P<0.001). Staining for cyclin B1 and E2F-1 was also increased in p16 positive tonsillar tumors (P<0.01).
Conclusions: E2F-1 and cyclin B1 are expressed in oropharyngeal squamous cell carcinomas along with p16. This previously unreported finding may indicate a role for cyclin B1 and E2F-1 in Human Papilloma Virus associated carcinogenesis and possible targets for therapy.

1261 Cadherin Expression Is Not Associated with Metastasis or Histological Grade in Oropharyngeal Squamous Cell Carcinomas

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Background: Cadherin expression has been shown to play an important role in metastasis in head and neck squamous cell carcinoma (SCC). Specifically, loss of E-cadherin and concomitant increase in N-cadherin expression promotes local invasion and metastasis. Human papilloma virus (HPV)- related oropharyngeal squamous cell carcinomas have a higher propensity to metastasize than their keratinizing-type counterparts. However, cadherin expression specifically in oropharyngeal SCC has not been examined. The purpose of this study was to evaluate E- and N-cadherin expression in oropharyngeal SCC and to correlate with clinical and pathological features.
Design: 123 oropharyngeal SCC patients with clinical follow up information were identified from an approved cancer database. Tumors were graded as follows: keratinizing (1), non-keratinizing (3), or hybrid/non-keratinizing with maturation (2). Immunohistochemistry was performed for p16 and graded 0 to 4. Immunohistochemistry was performed for E- and N-cadherin. Staining was graded according to presence and then intensity (0= No staining, 1= weak, 2= moderate, 3= strong).
Results: The tumors had the following histologic breakdown: Type 1: 27 (21%), Type 2: 23 (19%), Type 3: 73 (60%). E-cadherin expression was present in 123 (98%) of the specimens (no stain- 2%, weak- 9.5%, moderate- 55.1%, strong- 33.3%). N-cadherin expression was present in 17 (11.5%) of the specimens (no stain- 87.1%, weak- 9.5%, moderate- 2%). Neither E- nor N-cadherin expression was associated with histological grade (OR= 1.67 p=.082; OR=4.26 p =.228, respectively). E-cadherin staining grade was not associated with nodal or distant metastasis (OR= 8.50 p=.098; OR= 1.00 p=.963 respectively) nor was N-cadherin (OR= 4.26 p=.228; OR= 1.00 p=.935 respectively). Neither cadherin was associated with p16 expression, either. In follow up, expression of neither E- or N-cadherin was associated with death from disease (p = 0.995; p=.964, respectively).
Conclusions: Despite the numerous studies suggesting cadherin expression to be associated with metastases and outcome in head and neck SCC, our data suggests that it is not a predictor for nodal and distant metastasis in oropharyngeal SCC, specifically. Even with non-keratinizing (poorly differentiated) morphology, we still observed strong staining for E-cadherin. Our results suggest that mechanisms independent of cadherin expression must explain the early and frequent metastases that occur in oropharyngeal SCC.

1262 Prevalence of Human Papilloma Virus in Neck Nodal Metastatic Squamous Cell Carcinoma from Unknown Head and Neck Primary

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Background: Significant proportion of head and neck squamous cell carcinomas (HNSCC) metastasize to neck lymph nodes. Despite a complete workup, including extensive imaging modalities and panendoscopy with targeted biopsies, the primary tumor remains unknown in 5% of HNSCC. In such instances, wide-field radiation of the entire pharynx and larynx is usually undertaken resulting in increased morbidity. Ability to predict or identify primary site will therefore reduce therapy-associated morbidity. Human papilloma virus (HPV) is etiologically associated with oropharyngeal subset of HNSCC, particularly those arising from the tonsil which often has non-keratinizing basaloid features. This study aims to determine the prevalence of HPV+ nodal metastasis in our patient population and the associated morphologic features.
Design: Cases of cervical lymph node SCC from unknown primaries were identified from the institution Tumor Registry and PET database. The histological sections were reviewed, morphologically typed and graded; formalin fixed paraffin embedded tissue sections were cut and stained using the Ventana INFORM HPV III Family 16 (B) probe according to the manufacturer's instructions with appropriate positive and negative controls. Positive HPV staining is detected as dark blue punctuate and diffuse staining pattern within tumor cells nuclei.
Results: Twenty-seven cases of nodal SCC metastasis with suitable tissue blocks from unknown primaries were identified. Seven cases (26%) were found to be HPV+ tumor metastasis; morphologically six (6) of 7 cases showed basaloid non-keratinizing cystic squamous features.
Conclusions: The prevalence of HPV+ nodal metastasis is 26%. Inferring from our previous study, it is likely that metastatic HPV+ tumors are of oropharyngeal origin, since HPV+ primary SCC are more common in the oropaharynx. Hence we suggest that HPV+ metastatic tumor with non-keratinizing basaloid cystic features may be predictive of tonsillar or oropharyngeal origin in cases of unknown HNSCC primary.

1263 Significance of Human Papillomavirus in Head and Neck Squamous Cell Carcinoma

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Background: Head and neck squamous cell carcinoma (HNSCC) continues to be a significant disease with varying rates of incidence and mortality worldwide. Numerous studies have demonstrated that human papilloma virus (HPV) is etiologically linked with a subset of HNSCC, independent of tobacco and alcohol use. This subset of tumor show increased sensitivity to radiation therapy and association with better outcomes. However, the burden of HPV-positive HNSCC varies amongst population, in different geographic areas and by detection methods. The purpose of this study is to determine the HPV burden and significance amongst HNSCC patients in the southern region of the US.
Design: The study materials consist of formalin-fixed paraffin embedded tissue blocks of HNSCC cases seen at the Pathology Department in our institution between January 2000 and December 2005. The hematoxylin and eosin stained sections were reviewed, fresh 5 mm sections cut and stained with Ventana HPV Inform III probe using the ISH technique according to the manufacturer's instructions with adequate positive and negative controls. Positive signal is indicated by dark blue punctate and diffuse staining patterns within tumor cells nuclei.
Results: A total of 259 cases with HNSCC were recorded between 2000 and 2005. Of these 174 specimen blocks are available for review and 97 suitable specimens stained for HPV. The loco-regional distribution of these 97 cases is: oral cavity (42), larynx (28), oropharynx (20) including 5 tonsils, hypopharynx (5), and nasopharynx (2). Nine cases (9.4%) were positive for high risk HPV. Six of 9 cases were oropharyngeal tumors including 2 cases from the tonsil. These constitute 30% (6 of 20) of oropharyngeal tumors and 40% (2 of 5) of tonsillar tumors. The other 3 HPV+ cases were from the oral cavity (2) and larynx (1).

Conclusions: The overall 9.4% high risk HPV prevalence in 97 cases suggests a comparatively low HPV associated tumor burden in our patient population. Our results however support emerging evidence that HPV is strongly associated with oropharyngeal squamous cell cancer.

1264 Skull Base Chordoma: An Immunohistochemical (IHC) Study of 9 Cases Showing Differentiating Staining from Skull Base Chondrosarcoma

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Background: Chordoma is a malignant tumor arising from remnants of the notochord that is most common in the sacrococcygeal region followed by the craniocervical region/skull base and usually can be differentiated from chondrosarcomas by their pathologic and IHC findings, but these tumors may share overlapping findings creating difficulties in their differentiation, especially in biopsy material. Further, the origin for chondroid chordoma remains controversial whether it is a hybrid tumor with chordomatous and cartilaginous differentiation or is a chondrosarcoma variant. The goals of this study were to determine if there are differentiating IHC findings between these tumors and to try and address the issue of classifying chondroid chordoma.

Design: We identified 9 cases of skull base chordomas and 7 cases of skull base chondrosarcomas from our files over a 5-yr period (2005-09); the clinical and pathologic features were reviewed. IHC staining included cytokeratins (AE1/AE3, CAM 5.2), EMA, p63, brachyury, D2-40, S100 protein, vimentin, NSE, GAL-3, GFAP and VEGF.

Results: Chordomas included 6 females and 3 males ranging in age from 8 to 58 years (median, 48 years). These tumors include conventional chordoma (n=7), chondroid chordoma (n=1) and dedifferentiated chordoma (n=1). All chordoma subtypes were immunoreactive for cytokeratins (AE1/AE3, CAM5.2), EMA, S100 protein, brachyury, and vimentin, and were negative for D2-40. Chondrosarcomas included 5 males and 2 females ranging in age from 51-62 yrs (median 58 yrs). All chondrosarcomas were histologically low-grade (Grade I) and included one case of a clear cell type. The chondrosarcomas were immunoreactive for D2-40, S100 protein and vimentin but negative for epithelial markers and brachyury.

Conclusions: Based on our study, skull base chordomas have a distinct IHC antigenic profile contrasting with that of skull base chondrosarcomas. The chordomas consistently expressed epithelial markers and brachyury but were negative for D2-40 while the chondrosarcomas were consistently reactive for D2-40 but negative for epithelial markers and brachyury. Both tumor types expressed S100 protein and vimentin. The results of our findings provide a mechanism for differentiating chordomas from chondrosarcomas, affirm that chondroid chordoma appropriately be classified within the spectrum of chordomas and support the development of chordomas from the notochord.

1265 Differentiated Thyroid Carcinoma in Children and Adolescents: A Clinicopathologic Analysis of 67 Cases

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Background: Recently, the incidence of differentiated thyroid carcinoma (DTC), including papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC), has increased in children and young adults. As DTC's clinic, pathologic, and prognostic features in children and adolescences are different from that in adults, we report 67 case of DTC in children and young adolescence.

Design: Retrospective review and analysis of clinic, pathologic and follow-up results of 67 patients under 20 years old with DTC between 2000 to 2005. On review and analysis, we used the WHO classification of thyroid carcinoma to categorize the PTC and FTC.

Results: Among 67 cases, 15 were male and 52 female (M:F=1:3.5), of them, 8 of 4~<12 years, 31 of 12~<16, 28 of 16~20, with history of painless neck mass of 1 month to 6 years, 10 with increased neck lymph nodes as first sign, and 3 hoarseness. Pre-op color-Doppler ultrasound revealed solid lesion in 53 cases (79.1%), 14 with cystic changes and papillary projections, 27 with ipsilateral and 6 bilateral increased sizes of regional lymph nodes. No remote metastasis were identified. Pre-op FNA were performed in 49 cases, 39 suggestive for malignancy, and 18 cases with intra-op frozen section, 16 diagnosed malignancy. For surgical treatment: 12 had lobectomy with ischmus resection, 23 lobectomy with ischmus and opposite subtotal lobectomy, 32 radical resections. Of the 67 cases, pathological diagnosis were: PTC in 42, FTC in 17, PTC combined with FTC in 8. Lymph node metastasis were confirmed in 29 cases (43.3%), of them, 6 (9%) being bilateral thyroid carcinoma and bilateral node positive. All patients survived well and follow up with no recurrence or remote metastasis, except one patient had contralateral lymph node metastasis 2 years after surgery and underwent regional neck dissection.

Conclusions: DTC in children and adolescence shows non-specific symptom in clinic, and pre-operative diagnosis is difficult. FNA under the guidance of ultrasounde can reach a specific diagnosis in 83~91%, intra-operative frozen section can establish a definitive diagnosis, and help for selection of the correspondent surgical procedures. Short term follow-up shows the children and adolescence with DTC had early and therapeutic surgery had a good prognosis.

1266 Epigenetic Profiling of Cancer Stem Cells (CSC) in Head and Neck Squamous Cell Carcinoma (HNSCC)

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Background: A small population of pluripotent CSCs with strong self-renewal capacity will survive most forms of chemoradiation therapies. Current chemoradiation therapy regimens for HNSCC may selectively kill the differentiated cancer cells, producing tumor regression while sparing a very small population of cancer stem cells, leading to tumor regrowth and relapse. Molecular characteristics for HNSCC CSCs are poorly understood, which prohibits design of more effective anticancer therapies, specifically targeting the CSCs.

Design: CSCs were isolated in 5 HNSCC lines (HEp2, MDA1986, SQ-20B, T409, TU167) using CD44 fluorescent antibody with flowcytometric sorting. Total RNA was extracted from both CD44+ CSCs and CD44- non-SC from 5 HNSCC lines for stemness genes (CD44, BMI-1, TERT, SALL4 and ABCG) expression. Genomic DNA was extracted from both CD44+ CSCs and CD44- non-SC from 5 HNSCC lines for epigenetic profiling using Illumina BeadArray (Human Methylation27), representing a total of 14,956 genes and for pathway analysis using ArrayTrack software.

Results: CD44+ CSCs expressed significantly higher levels of majority of stemness genes in all 5 HNSCC lines. With methylation of at least twice as much in CD44+ CSCs in most of the 5 HNSCC lines, we selected 22 genes that may be functionally very significant in head and neck CSCs. Cluster analysis using these 22 genes showed that CD44+ CSCs were epigenetically distinct from the CD44- Non-SCs in HNSCC. By ArrayTrack analysis, we further identified a group of 10 among 22 genes that are common in their metal ion binding capability. The difference in methylation pattern of these 10 genes in CSCs is statistically very significant ($p = 0.0009$) as compared to that seen in the non-SCs.

Conclusions: A small population of CD44+ CSCs was present in HNSCC that possess a unique epigenetic signature. The 10 genes identified by methylation microarray and ArrayTrack may be functionally significant in maintaining the stem cell property and thus could represent novel molecular targets for design and development of more effective anticancer therapies aiming specifically at these CSCs.

Hematopathology

1267 Cyclin D1 Positive Diffuse Large B-Cell Lymphomas Feature a Post-Germinal Center – Immunophenotype and Lack Alterations in the CCND1 Gene Locus

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Background: Diffuse large B-cell lymphomas (DLBCL) are a heterogeneous group of aggressive lymphomas and are generally believed to be cyclin D1 negative. However, in the last years there have been some reports of DLBCLs expressing cyclin D1. The association of cyclin D1 expression with specific histological subtypes of DLBCLs and/or in the setting of Richter´s transformation has not been studied systematically. The aims of this study were to analyse the incidence of cyclin D1 overexpression in DLBCLs and Richters transformation (RT) cases and to elucidate the possible molecular mechanism.

Design: Seventy-six cases of DLBCLs, including 67 de novo DLBCL and 9 RT cases were included in this study. Immunohistochemical stainings for CD20, CD5, CD30, BCL-2, BCL-6, CD10, MUM1, cyclin D1and Ki.67 were performed and complemented by immunofluorescence double stainings. CCND1 and c-MYC gene loci were analyzed by FISH.

Results: Of the 67 de novo DLBCLs, 13 cases (19.4%) were cyclin D1+ in >10% of the neoplastic cells. Immunofluorescence double stainings demonstrated cyclin D1 positivity in CD20+ tumor cells. Only one case of RT was cyclin D1+ (11%). To better characterize the cyclin D1+ DLBCL, seven cyclin D1+ DLBCL from other institutions were included in the analysis. The 21 cyclin D1+ DLBCL cases (20 de novo and 1 RT) displayed heterogeneous morphological patterns. All cases were negative for CD10. Bcl-6 was positive in 20 of 21 cases and MUM1 was positive in 11/14 cases. Only two cases were partially CD5+. No CCND1 gene locus alterations were identifed by FISH analysis, except for one case. No c-MYC translocations were identified.

Conclusions: 1) Cyclin D1 expression in DLBCL is not associated with a particular morphology but consistently shows a post-germinal or activated B-cell phenotype (CD10-, BCL-6+, MUM1+). 2) The abnormal expression of cyclin D1 is not associated with a t(11;14) or alterations in chromosome 11, suggesting an alternative mechanism of cyclin D1 deregulation.

1268 Flow Cytometric Characterization of Peripheral Blood CD34+ Cells in Patients with Primary Myelofibrosis

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Background: Primary myelofibrosis (PMF) is a chronic myeloproliferative neoplasm characterized by the accumulation of abnormal megakaryocytes in the bone marrow (BM), variable degrees of BM fibrosis, tear-drop erythrocytes, circulating nucleated red blood cells with increased CD34+ blasts, and extramedullary hematopoiesis. The antigenic characteristics of circulating CD34 + cells in PMF may yield clues to disease pathogenesis and/or diagnosis, but have not been extensively studied.

Design: Peripheral blood CD34+ cells from 15 well characterized PMF patients and 10 healthy controls were examined by 5-color flow cytometry using a large panel of antibodies. Bone marrow biopsies, molecular and cytogenetic studies, and clinical parameters were also reviewed and correlated with the flow cytometry findings.

Results: As expected, the percentages of peripheral-blood CD34 cells were significantly higher in the PMF patients (mean 1.38%, range, range 0.065-7.15) compared to the controls (mean 0.05%, range 0.01- 0.57). All but one PMF case showed phenotypic abnormalities on the CD34+ cells with 5/15 cases having 2 abnormalities, and 2/15 having ≥3 . Abnormalities included increased mean fluorescence intensity (MFI)