of cases from each of the different SCST categories have compared the utility of the more commonly used older SCST markers with ones that have been utilized more recently.
**Design:** IHC stains for WT1, SF1, Mart-1, Inhibin, Calretinin, and CD99 were performed in 127 cases from 5 different categories of ovarian SCSTs: Sertoli cell tumor (SertCT), adult granulosa cell tumor (AGCT), steroid cell tumor (SterCT), Sertoli-Leydig cell tumor (SLCT), and fibroma/fibrothecoma (F/FT). Extent of staining was based on the percentage of positive cells: 0, <5%; 1+, 6-25%; 2+, 26-50%; 3+, 51-75%; and 4+ 76-100%. Intensity of staining was scored as 1+, 2+, or 3+. IHC composite scores=extent score x intensity score.
**Results:** Table 1 and Table 2.

Table 1. Overall Positivity

| Tumors | n | WT1 | SF1 | Mart-1 | Inhibin | Calretinin | CD99 |
|---|---|---|---|---|---|---|---|
| SertCT | 27 | 100% | 100% | 0% | 96% | 48% | 59% |
| AGCT | 32 | 78% | 100% | 0% | 94% | 81% | 88% |
| SterCT | 25 | 0% | 100% | 96% | 100% | 100% | 32% |
| SLCT (SC) | 18 | 100% | 100% | 0% | 94% | 44% | 50% |
| SLCT (LC) | 18 | 0% | 100% | 94% | 100% | 100% | 6% |
| F/FT | 25 | 100% | 100% | 0% | 56% | 36% | 0% |

SC, Sertoli cell component; LC, Leydig cell component.

Table 2. IHC Composite Scores*,**,***

| Antigen | SertCT | AGCT | SterCT | SLCT (SC) | SLCT (LC) | F/FT |
|---|---|---|---|---|---|---|
| WT1 | 10.8 (4-12) | 4.2 (1-12) | - | 10.4 (6-12) | - | 8.9 (3-12) |
| SF1 | 6.1 (1-12) | 6.2 (1-12) | 10.1 (2-12) | 9.9 (1-12) | 8.9 (1-12) | 8.3 (2-12) |
| Mart-1 | - | - | 8.6 (1-12) | - | 7.9 (2-12) | - |
| Inhibin | 7.8 (1-12) | 10.0 (4-12) | 11.2 (8-12) | 6.5 (2-12) | 11.8 (9-12) | 4.3 (1-9) |
| Calretinin | 7.2 (1-12) | 5.6 (1-12) | 10.8 (4-12) | 6.4 (2-12) | 10.8 (2-12) | 3.4 (2-6) |
| CD99 | 4.7 (1-12) | 8.6 (1-12) | 8.6 (6-12) | 6.3 (1-12) | 2* | - |

*, Scores calculated only for positive cases; **, Results reported as: Mean (range); ***, Only one case positive.
**Conclusions:** SF1 is the most diagnostically sensitive SCST IHC marker, and some markers are slightly more sensitive than others for detecting sex cord-stromal lineage. Within each different category of SCST, there are slightly unique differences in terms of which markers are more sensitive than others. Mart-1 is particularly sensitive and specific for SterC and SLCT.

### 1052 SF1 Is Diagnostically Useful and Comparable to Other Sex Cord-Stromal Tumor Immunohistochemical Markers for the Differential Diagnosis of Ovarian Sertoli Cell Tumor

*C Zhao, TN Vinh, K McManus, D Dabbs, R Vang.* Magee-Womens Hospital, University of Pittsburgh, Pittsburgh, PA; Armed Forces Institute of Pathology, Washington, DC; Johns Hopkins Hospital, Baltimore, MD.
**Background:** Steroidogenic factor 1 (SF1, Ad4-Binding protein) is a transcription factor that regulates the expression of steroidogenic enzymes. SF1 has been shown to be expressed in ovarian sex cord-stromal tumors; however data is limited. Expression in ovarian pure Sertoli cell tumor (SCT) and potential application for the differential diagnosis of SCT have not been studied.
**Design:** Immunohistochemical staining for SF1 (Clone N1665, R&D System), Inhibin (Clone R1, Dako), and WT1 (Clone Cell Marque) was performed in 111 ovarian tumors: pure SCT, endometrioid borderline tumor, sertoliform endometrioid carcinoma, well-differentiated endometrioid carcinoma, and carcinoid. Extent of staining was based on the percentage of positive cells: 0, <5%; 1+, 6-25%; 2+, 26-50%; 3+, 51-75%; and 4+ 76-100%. Intensity of staining was scored as 1+ (weak), 2+ (moderate), or 3+ (strong). Immunohistochemical composite scores were calculated (extent score x intensity score).
**Results:** See table 1 and 2.

TABLE 1. Overall Positivity for SF1, WT1, and Inhibin

| Antigen | Sertoli cell tumor (n=27) | Endometrioid borderline tumor (n=25) | sertoliform endometrioid carcinoma (n=12) | well-differentiated endometrioid carcinoma (n=23) | Carcinoid (n=24) |
|---|---|---|---|---|---|
| SF1 | 100% | 0 | 0 | 0 | 0 |
| WT1 | 100% | 16 | 25 | 13 | 0 |
| Inhibin | 96% | 0 | 0 | 4 | 0 |

TABLE 2. Extent and Immunohistochemical Composite Scores of Expression of SF1, Inhibin, and WT1 in Sertoli Cell Tumor (n=27)

| | | | Extent | | | IHC Comp Score* | |
|---|---|---|---|---|---|---|---|
| Antigen | 0 | 1+ | 2+ | 3+ | 4+ | Mean | Range |
| SF1 | 0 | 11% | 26% | 33% | 30% | 6.1 | 1-12 |
| WT1 | 0 | 0 | 4% | 7% | 89% | 10.8 | 4-12 |
| Inhibin | 4% | 4% | 15% | 22% | 55% | 7.8 | 1-12 |

*, Immunohistochemical composite score (calculated only for positive cases).
**Conclusions:** SF1 is a sensitive immunohistochemical marker for ovarian SCT and helpful for distinction from other non-sex cord-stromal tumors in the differential diagnosis. The diagnostic usefulness of this marker is comparable to that of inhibin and WT1.

### 1053 Expression of a Panel of Immunohistochemical Stains in Normal Cervix, Endometrium, and Uterine Carcinomas

*J Zhou, A Khiyami, J Tomashefsky, K Zilch, J Sawady.* MetroHealth Medical Center, Case Western Reserve University, Cleveland, OH.
**Background:** The diagnosis of endometrial carcinoma can be difficult, especially in small surgical biopsy samples. A panel of immunohistochemical stains, including the new marker PK-M2, was performed in a variety of normal and neoplastic uterine tissue, to assess its diagnostic utility.
**Design:** Monoclonal antibodies against vimentin, EMA, PK-M2, and p53 using the Vector Immpress Mouse kit (Burlingame, CA 94010) were performed in 57 paraffin-embedded specimens including 28 endometrial carcinomas, 5 endometrial polyps, 9 samples of normal endometrium, 9 samples of normal endocervical tissue, and 6 endocervical carcinomas. Results were recorded as negative (-), weakly positive (+), positive (++), and strongly positive (+++). The results were analyzed statistically using chi-square test.
**Results:** See Table 1 for a summary of the results.

Table 1. Immunohistochemical expression of different markers in study samples.

| | Vimentin* | EMA* | PK-M2* | P53* |
|---|---|---|---|---|
| Endometrial | | | | |
| Benign | 14/14 (100%) | 14/14 (100%) | 14/14 (100%) | 3/14 (21.4%) |
| Malignant | 27/28 (96.4%) | 27/28 (96.4%) | 26/28 (92.9%) | 14/28 (50%) |
| Endocervical | | | | |
| Benign | 0/9 (0%) | 5/9 (55.5%) | 9/9 (100%) | 0/9 (0%) |
| Malignant | 1/6 (16.7%) | 5/6 (83.3%) | 6/6 (100%) | 1/6 (16.7%) |

* including +, ++, and +++
Significant findings include: (1) 96.4% (27/28) of endometrial carcinomas were positive for vimentin, compared to 16.7% (1/6) of endocervical carcinomas ($p < 0.001$). (2) 83.3% of endocervical carcinomas showed strong positivity in EMA expression, compared to 0% (0/9) of normal endocervical glands with strong positivity ($p < 0.001$). 55.5% (5/9) of normal endocervical glands demonstrated weakly positive or positive stain on EMA. In addition, only 17.9 % (5/28) endometrial carcinomas were strongly positive for EMA. (3) Endometrial carcinomas were weakly positive (32.1%) or negative (7.1%) in PK-M2 expression, in contrast to benign endometrium (all cases with strong positivity) ($p < 0.05$). (4) 50% of endometrial carcinomas were positive for P53 as opposed to 11.1% (1/9) of normal endometrial samples ($p < 0.05$). In addition, 2 out of 5 endometrial polyps were also weakly positive for P53.
**Conclusions:** A panel of immunohistochemical stains including vimentin, EMA, PK-M2, and P53 can be used in difficult small biopsies to help differentiate endometrial carcinoma from endocervical carcinoma, and/or their benign counterparts.

# Head & Neck

### 1054 Angiogenesis, MIB 1, DNA Ploidy and p53 in Mucosa Adjacent to Oral Squamous Cell Carcinomas

*N Aggarwal, SG Sharma, SD Gupta, AK Dinda, BK Mohanty, MK Singh.* All India Institute of Medical Sciences, Delhi, India.
**Background:** Histopathology is gold standard to determine completeness of surgical therapy in oral squamous cell carcinoma (OSCC). However, the status of the histologically benign area adjacent to tumor (shoulder) is unknown. We studied p53 expression, MIB 1, DNA ploidy and angiogenesis in OSCCs, shoulder and normal mucosa.
**Design:** This is a prospective study on surgical resection specimens from 33 patients of OSCC. We compared the tumor, shoulder (1 to 2 cms) and normal oral mucosa for angiogenesis by counting microvessel density (MVD) on CD34 immunohistochemical (IHC) stained slides and DNA ploidy (measuring integrated optical density on fuelgen stained slides) by computerized image analysis system. MIB 1 and p53 expression were evaluated on IHC stained slides. p53 was considered to be overexpressed if > 10% of the cells showed nuclear staining. Non parametric tests were used for statistical analysis. The clinical follow up is pending.
**Results:** The study population consisted of 27 males and 7 female, mean age was 49.81 yrs (30-70 yrs). 27/33 patients consumed tobacco. The sites involved were buccal mucosa (5/33), gingiva (14/33), anterior 2/3$^{rd}$ of the tongue (12/33) and lip (2/33). The TNM stage of tumors was stage 1 (7/33), stage 2 (7/33), stage 3 (2/33) and stage 4 (17/33). The tumor showed a higher MIB1 and p53 expression as compared to the shoulder region and normal area (p value <0.05). The staining for MIB 1 and P53 was restricted to the basal cells in normal mucosa, extended to suprabasal cells in the shoulder and was diffuse or along the infiltrating edge in the tumor. p53 overexpression was seen in 17/33 (51.5%) of the tumors and 8/31 (25.51%) of the shoulder. Except for one, tumor in all the cases showed aneuploid cell lines. The shoulder region had aneuploid cell lines in 50% of cases while all normal mucosae were diploid. The difference in MVD between the tumor (median 143.48 vessels/mm$^2$), shoulder region (median 147.82 vessels/mm$^2$) and normal mucosa (median 163.04 vessels/mm$^2$) was not siginificant ($p = 0.28$). Correlation of MVD, p53 and MIB1 within tumor was not significant.
**Conclusions:** The pattern of p53 and MIB-1 expression, coupled with presence of aneuploid cell lines in the shoulder suggest that this area may be significantly abnormal though it appears histologically benign. This field effect may have a clinical impact in terms of local recurrence. Angiogenesis was not significantly increased in tumor making the role of antiangiogenic drugs questionable.

### 1055 Nodular Fasciitis of the Salivary Gland. A Clinicopathologic Study of 14 Cases

*W Al-Daraji, ELB Childers, JC Fanburg-Smith.* Armed Forces Institute of Pathology, Washington, DC; Howard University College of Dentistry, Washington, DC.
**Background:** Nodular fasciitis of the salivary gland is rare; there are only 13 cases total reported to date, all in the parotid gland. We reviewed our clinicopathologic experience with salivary gland nodular fasciitis.
**Design:** Cases coded as "nodular fasciitis" in all salivary glands were culled for clinicopathologic review.
**Results:** There were 14 cases from 1971-2003, 7 males and 7 females. Patient ages ranged from 4-51 (median 31.5) years; only 2 were less than 20 years. No patients recalled antecedent trauma. Symptoms ranged from rapid to one year of a painless to painful mobile mass, 2 "near the facial nerve." There were no changes of overlying skin. One patient had trismus. 10 cases were in the parotid gland (5 right, 5 left sided); 4 in submandibular gland (3 left, 1 right). Tumor size ranged from 1-4 (median 1.5) cm in greatest dimension. All but 1 case were well-circumscribed and had alternating spindled tissue culture like myoifibroblasts with myxoid degeneration and extravasated erythocytes and lymphocytes. Three cases demonstrated stromal keloidal collagen.

Osteoclast type giant cells were not observed. Increased mitotic activity was observed in 1 case. Of cases studied by immunohistochemistry, lesional cells were positive for SMA, 1 case for MSA, and negative for desmin, keratins, S100 protein, and CD34. Original diagnostic considerations varied from nodular fasciitis, myxoma and fibrous histiocytoma to fibromatosis, and rule out leiomyosarcoma. Complete lesional excision to partial parotidectomy were performed. No cases had recurrences up to 7 years follow-up, even with incomplete excision.
**Conclusions:** Nodular fasciitis, a benign reactive lesion, is a diagnostic consideration for spindle cell lesions in the parotid and also submandibular gland, particularly in adult patients with a tender to non-tender non-ulcerative mobile mass in these areas. It should not be histologically confused with malignancy; it does not recur in this location. Generally these patients do not recall antecedent trauma, unlike nodular fasciitis of soft tissue sites.

### 1056 Dysplasia and Carcinomas Arising in Schneiderian Papillomas, 20 Years Experience

*DS Allende, A Hoschar, P Batra, JL Hunt.* Cleveland Clinic, Cleveland, OH.
**Background:** The reported incidence of carcinomas in inverted papillomas varies between 2 and 27%. Differentiating in situ from invasive lesions can be difficult at the histologic level. Furthermore, the molecular alterations in the dysplasia-carcinoma pathway are incompletely understood.
**Design:** 170 schneiderian lesions from the sinonasal tract collected during 1986-2006. Clinical information was obtained. Lesions were divided in 3 groups: schneiderian papillomas, schneiderian papillomas with dysplasia and schneiderian papillomas with carcinoma. Detailed pathologic analysis was performed. Immunohistochemistry for p53, HPV, and p16 were evaluated on all available cases of dysplasia and carcinoma, and a subset of typical inverted papillomas.
**Results:** 153 were inverted papillomas (91%), 6 had moderate to severe dysplasia (3%) and 11 had carcinoma (6%). 3/6 patients with dysplasia and 5/7 patients with carcinomas had recurrences. Several features were identified that helped differentiate invasion from inverted growth, including loss of transmigrating neutrophils, desmoplastic stromal reaction and a variable size and shape of nests in the stroma. 4/7 inverted papillomas were focally positive for p16 and 7/7 were negative for p53. 2/6 dysplastic lesions showed patchy staining for p16 and only 1/6 was positive for p53. 2/7 carcinomas were positive with p16 and 6/7 positive for p53. HPV was negative in all cases.
**Conclusions:** Carcinomas and dysplasias are an infrequent finding in schneiderian papillomas. Loss of transmigrating neutrophils, desmoplastic stroma, and variable size and shape of stromal nests helped differentiate in situ from invasive lesions. p53 is involved in the dysplasia-carcinoma pathway. Most dysplasias and carcinomas were negative for p16.

### 1057 Exophytic Schneiderian Papillomas and Their Differential Diagnosis: Can p16 Help in the Diagnosis of These Two Entities?

*DS Allende, A Hoschar, JL Hunt.* Cleveland Clinic, Cleveland, OH.
**Background:** The exophytic (fungiform) subtype of inverted papilloma can be difficult to differentiate from cutaneous-derived papillary lesions (squamous papillomas and verruca). They can arise in the same location and have a similar gross appearance. Differential diagnosis can be challenging, especially in small biopsies. An association with HPV has been suggested, but is not completely well understood.
**Design:** 64 nasal septal lesions from the Cleveland Clinic files from 1986 to 2006 were studied. Age, gender and anatomic location information was collected. Immunohistochemistry for HPV and p16 was performed in a subset of 20 of each lesion.
**Results:** There were 42 squamous papillomas located in the nasal area. They appeared in patients from 28-79 years with a male predominance (62%). There were 22 exophytic schneiderian papillomas (59% males). The age range was 25 to 82 years. Histologically, both lesions have a papillary growth pattern, with squamous-type epithelium. Exophytic schneiderian papillomas have occasional transitional and/or respiratory-type epithelium in most lesions, and some of them have goblet cells. One was associated with a carcinoma (4%). In the cutaneous squamous papillomas, p16 was strongly and diffusely positive in 7/20, and was focally and weakly positive in 8; 5 were negative. In exophytic papillomas, 10 were strongly positive, 2 were focally positive and 8 were negative. HPV was negative in all cases.
**Conclusions:** The differential between exophytic Schneiderian papilloma and squamous papilloma is only possible at the histologic level. It relies on the finding of transitional epithelium and/or goblet cells. p16 and HPV do not help to differentiate these lesions.

### 1058 A Complete Histopathologic and Clinical Analysis of Sinonasal Schneiderian Papillomas. A Study of 188 Cases

*DS Allende, A Hoschar, JL Hunt.* Cleveland Clinic, Cleveland, OH.
**Background:** Scheneiderian papillomas constitute 0.5 to 4 % of all nasal lesions. Several studies suggested variable morphology in this lesions and this can contribute to diagnostic challenges. No studies have presented a thorough histologic description to help understand the spectrum of this entity.
**Design:** 188 cases were reviewed from the files of Cleveland Clinic from 1986 to 2006. Epidemiologic and histologic characteristics were evaluated in each lesion. Dysplastic lesions or carcinomas were excluded from this study.
**Results:** Cases were divided in 4 groups: inverted papillomas NOS with mixed transitional and squamous epithelium (n:153), fungiform papillomas (n:22), oncocytic (n:13). Inverted papillomas were more common in men (80 %). Most were in the nasal cavity (59%). Inverted papillomas were composed mostly or exclusively of transitional epithelium (80%), with 10 cases being exclusively squamous. All of them showed a variable degree of acute inflammation. In 67% of the cases a respiratory cap was found overlying the transitional epithelium. Goblet cells were found consistently in lesions from the septum (n:23), and 48% of them have an inverted growth pattern.
**Conclusions:** Inverted papillomas were the most frequent subtype of scheneiderian papillomas and most were transitional type. Respiratory epithelium was almost exclusively seen associated with transitional type. Goblet cells were only seen in septal lesions, interestingly almost half of them were from the inverted type. Squamous epithelium as a major component was present only in a minority of cases. Understanding the morphologic spectrum of Schneiderian papillomas will help in difficult differential diagnoses.

### 1059 Differentiation of Follicular Lesions of the Thyroid Based on Gene Expression Analysis

*ME Barcus, DW Bigwood, EM Eastman.* Gene Logic Inc., Gaithersburg, MD.
**Background:** Fine needle aspiration biopsy (FNAB) has proven utility in the evaluation of thyroid nodules, but definitive classification of follicular lesions is not possible by cytomorphology alone. Given that these lesions merit distinct therapy and that there is significant morbidity associated with thyroid surgery, there is a clear benefit to achieving a diagnosis preoperatively. We have evaluated whether gene expression analysis may clarify aspects of the pathogenesis of these lesions and allow sufficient discrimination to enable improved surgical management based on FNAB.
**Design:** Genome-wide gene expression analysis was performed on eleven follicular neoplasms (8 adenomas and 3 carcinomas). The neoplastic component in 9 of 11 samples represented ≥90% of the tissue analyzed, with tumor comprising 50-75% and 75-90% of the tissue for the remaining two samples. Total RNA was extracted using standard procedures and RNA quality (purity and integrity) was assessed using the Agilent Bioanalyzer and spectrophotometric analysis. Gene expression analysis was performed using Affymetrix HGU133 Plus 2.0 GeneChip® microarrays.
**Results:** Initial analysis identified a set of 872 gene fragments that are differentially expressed (raw p-values less than 0.05) between follicular carcinomas and follicular adenomas. When requiring the dysregulation to be at least two fold, the number of gene fragments is 324 (fold changes as high as ten-fold). We have identified various subsets of these gene fragments that discriminate between these two tumor types. Two of the top five most significant genes in this list are known to be involved in apoptosis and have higher expression in the carcinomas. Together, these two genes provide excellent separation between the follicular adenomas and carcinomas when a PCA analysis is performed.
**Conclusions:** Gene expression analysis allows discrimination of follicular lesions of the thyroid gland. Specifically, follicular adenomas and carcinomas may be differentiated on the basis of differential expression of two apoptosis-regulating genes. Although evaluation of greater sample sets is warranted, the pattern of up-regulation within these neoplasms does support the veracity of this early finding. It is expected that application of this process to FNAB material may allow definitive diagnosis preoperatively and optimize therapy.

### 1060 Basaloid Squamous Cell Carcinoma of the Head and Neck Is a Mixed Variant That Can Be Further Resolved by HPV Status

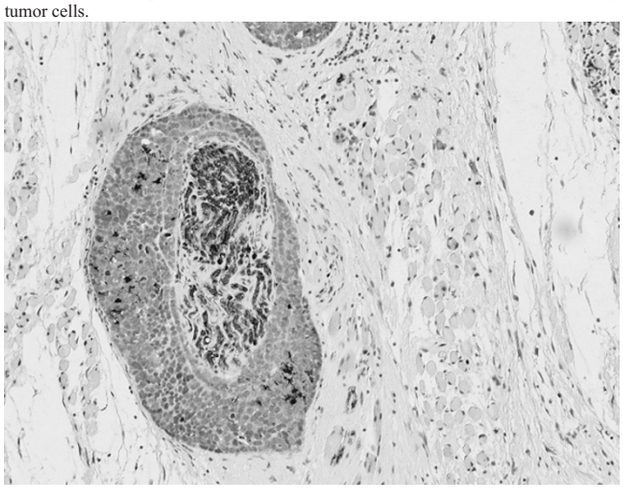
*S Begum, WH Westra.* The Johns Hopkins Medical Institutions, Baltimore, MD.
**Background:** Basaloid squamous cell carcinoma (BSCC) of the head and neck is set apart as a distinct subtype of squamous cell carcinoma based on its basaloid appearance and aggressive behavior. The purpose of this study was to determine whether BSCC could be further subdivided on the basis of HPV16 status.
**Design:** HPV16 in situ hybridization analysis and p16 immunohistochemistry was performed on 53 BSCCs of the head and neck.
**Results:** Of the 53 BSCCs, 21 (40%) arose in the oropharynx and 32 (60%) arose in extra-opharyngeal sites. HPV16 was detected in 36% of the BSCCs overall, but the frequency varied by site. HPV16 was detected in 17 of 21 (81%) BSCCs of the oropharynx, but in only 2 of 32 (6%) BSCCs from non-oropharyngeal sites ($p < .0001$). All 17 HPV16-positive oropharyngeal carcinomas exhibited p16 overexpression, whereas only 1 of the 4 HPV16-negative oropharyngeal tumors were p16 positive (100% vs. 25%, $p = 0.003$). For those BSCCs arising outside of the oropharynx, p16 overexpression was present in the 2 HPV16-positive tumors and in 7 of the 30 HPV16-negative tumors (100% vs. 23%, $p = .07$).
**Conclusions:** Morphologic similarities aside, BSCCs is comprised of a mixed group of tumors that can be separated on the basis of HPV16 status. The distinction is important. HPV16-positivity in squamous cell carcinomas of the head and neck is now recognized as a powerful indicator of improved patient survival. HPV16 detection thus permits resolution of a less aggressive component within a high grade subtype of head and neck carcinoma.

### 1061 S-100: Assisting in the Identification of Microscopic Perineural Invasion in Head and Neck Squamous Cell Carcinomas

*AR Bhalodia, BK Painter, A Abreo, P Adeboyega.* Louisiana State University, Shreveport, LA.
**Background:** In squamous cell carcinomas of the head and neck region (HNSCC), perineural invasion (PNI) has been associated with a poor outcome, including an increase in recurrence and a decrease in patient survival. The propagating tumor cells within or around perineural spaces may be difficult to identify in routine hematoxylin and eosin (H&E) staining. This study utilizes S-100, an immunoperoxidase stain that highlights cells of schwannian origin, to aid in the identification of potentially overlooked PNI in HNSCCs.
**Design:** Eighteen cases of post-operative recurrent HNSCC were selected. Of these, cases with positive resection margins, extracapsular lymph node extension, or perineural invasion at the time of surgery were excluded from the study. Eight cases of previously untreated HNSCC with negative resection margins and no evidence of

lymph node metastases or reported perineural invasion were studied. Ten paraffin blocks (1-2/tumor) were stained with the schwannian cell marker S-100. The presence of previously undetected microscopic PNI was recorded and the corresponding H&E slides were re-reviewed.

**Results:** PNI was reported in 0% (0/8) cases of tumors in the original reports. S-100 immunoperoxidase staining highlighted microscopic PNI in 63% (5/8) of those cases of HNSCCs. A majority of the cases showed tumor cells surrounding the nerves in an onion-skin configuration. One case showed microscopic intraneural invasion by tumor cells.



However, on a re-review of the cases, the neoplasms had extensive desmoplastic reaction associated with the locally advancing tumor cells, and PNI was difficult to appreciate on the corresponding H&E slides.

**Conclusions:** (1) PNI is an important prognostic indicator for patients with HNSCCs, and therefore should be thoroughly searched for in these tumors. (2) Routine H&E stains may show extensive stromal tissue responses that may mask PNI (3) Immunohistochemical enhancement with S-100 greatly improves the accuracy of detecting PNI in HNSCCs.

### 1062 Multinucleated Giant Cells Incidence, Immune Markers, and Significance: A Study of 172 Cases of Papillary Thyroid Carcinoma

*EG Brooks, L Simmons-Arnold, MF Evans, A Elhosseiny.* University of Vermont/Fletcher Allen Health Care, Burlington, VT.

**Background:** Multinucleated giant cells (MNGs) are often detected in cases of papillary thyroid carcinoma (PTC). Their origin and significance, however, has not yet been established. One possibility is that they form in response to the injury induced by recent fine needle aspiration biopsy (FNAB). Other hypotheses are that the chemically-altered colloid produced by PTC induces MNGs to act as colloidophages, or else the MNGs are a non-specific immune response to the carcinoma itself, ingesting neoplastic follicle cells.

**Design:** An average of 4 hematoxylin and eosin stained slides for each of 172 PTC study cases were assessed for the presence of MNGs. A semi-quantitative score was assigned as follows: none, few (1-2 MNGs), many (≥ 3 MNGs). Sections were cut from the formalin-fixed paraffin-embedded cases with "many" MNGs. To assess for MNG epithelial vs. histiocytic differentiation, sections were immunohistochemically stained with antibodies against AEI/AEIII keratins, CD68, and CD163. To assess MNG ingestion of colloid or thyroid follicle cells, sections were stained for thyroglobulin and TTF-1 respectively.

**Results:** MNGs were identified in 100/172 (58.1%) PTC specimens; in 45 (26.2%), "many" MNGs were found, while in 55 (31.9%) MNGs were "few." The average age of patients with numerous as opposed to rare/none MNGs was 42.2 (range 20-82) vs. 48.0 (range 9-82) years. The mean sizes of PTC in cases with many as opposed to rare/none MNGs was 2.50 cm (range 0.5-9.0 cm) vs. 1.76 (range 0.01-5.2 cm) [P=0.0025]. The cases of PTC with many MNGs had higher multifocality (26/45 vs. 51/127 [P=0.055]), extrathyroidal extension (21/45 vs. 36/127 [P=0.028]), and recurrence (8/45 vs. 9/127 [P=0.076]), than did cases with rare or no MNGs. The majority of patients both with and without numerous MNGs had previous histories of FNA or hemilobecotmy: 41/45 and 99/127 respectively (P=0.073). Immunohistochemistry performed on cases of PTC with numerous MNGs revealed the majority of MNGs to be positive for CD68 (45/45), CD163 (44/45), thyroglobulin (34/45) and negative for AEI/AEIII (44/45) and TTF-1 (44/45).

**Conclusions:** To our knowledge, this study of PTC from172 patients represents the most comprehensive analysis of MNGs to date. The results indicate that MNGs in PTC are of histiocytic origin. Cases of PTC with many MNGs have a significantly greater likelihood of extrathyroidal extension and greater tumor size than cases with few/no MNGs. MNGs appear to be functioning largely as colloidophages.

### 1063 Human Papillomavirus Is Common among Laryngeal Carcinomas but Appears Not To Be a Significant Etiogical Factor or Prognostic Marker

*EG Brooks, MF Evans, C Adamson, W Trotman, K Cooper.* University of Vermont/Fletcher Allen Health Care, Burlington, VT.

**Background:** Although alcohol and tobacco abuse are well-known risk factors for laryngeal squamous cell carcinoma, human papillomavirus (HPV) has also been suggested to have an etiological role. However, HPV detection rates in laryngeal carcinoma as well as estimates of HPV prognostic significance vary widely. Conflicting data may be attributable to small sample size or to the practice of grouping laryngeal carcinomas with other head and neck squamous cell carcinomas (HNSCCs).

**Design:** A cohort of 125 consecutive formalin-fixed, paraffin-embedded laryngeal squamous cell carcinoma hemilaryngectomy/laryngectomy specimens were collected from 125 patients. Purified DNA extract was quantified and tested for HPV by two different PCR assays utilizing GP5+/6+ primers. $P16^{INK4a}$ immunohistochemistry (IHC) was performed using clone JC8 (LabVison).

**Results:** The male to female ratio was 34:91, with age at diagnosis ranging from 34-93 (average: 63.6). Almost all patients had a history of tobacco abuse:123/125 (98.4%). In addition, 26 had a history of alcohol abuse (20.8%). Laryngeal carcinomas were localized as follows: 71 supraglottic, 37 glottic, 3 subglottic, and 14 involving more than one region. Forty-seven of the samples (38.8%) were HPV positive. All the HPV infections were high-risk types (i.e. HPV16,18,31,35), apart from one HPV11 case. Twenty-six (21.5%) specimens stained positive after p16 IHC. However, only nine samples were HPV positive both by PCR and p16 IHC (P=0.657). Of the HPV positive cases, 15/47 (31.9%) squamous cell carcinomas recurred and/or metastasized compared to 29/74 (39.2%) HPV negative cases (P=0.445). Six HPV positive cases were well-differentiated SCCs (12.8%) and forty-one (87.2%) moderate to poorly differentiated vs. eight well-differentiated (10.8%) and sixty-six (89.2%) moderate to poorly differentiated HPV negative SCCs (P=0.776).

**Conclusions:** Although 38.8% of laryngeal carcinoma patients tested HPV positive by PCR, HPV may not be a significant etiologic factor in these tumors. HPV status did not correlate with p16 staining, or with tumor grade or recurrence. These data contrast with other HPV associated HNSCCs (tonsillar/oral), where HPV defines a biologically distinct tumor subset. The data do not support HPV status as a useful prognostic marker of laryngeal tumors. HPV infections appear to be incidental, whereas tobacco/alcohol abuse are most likely the overriding risk factors for laryngeal carcinoma.

### 1064 Reproducibility of Our Histological Risk Assessment Model

*D Broughel, N Schlecht, A Negassa, M Ramer, B Wang, G Levy, M Prystowsky, M Brandwein-Gensler.* Montefiore Medical Center, Bronx; Albert Einstein College of Medicine, Bronx; Mount Sinai School of Medicine, NY; NYU Medical Center, NY; Beth Israel Medical Center, NY.

**Background:** We believe that the histologic risk model for head and neck squamous cell carcinoma (HNSCC) (1) can be applied to surgical pathology reporting, much like Gleason or Bloom-Richardson scores. To evaluate the reproducibility of risk scoring, we developed an educational course for surgical pathologists. Here we present data on the reproducibility of risk scoring from multiple observers.

**Design:** Training materials were developed showing key features for assessing the risk score (see www.aecom.yu.edu/pathology/search_programs.aspx?id=302). Pathologists all scored the same cases and determined risk category (low, intermediate, high risk). Feedback from the first training session (Phase I) was used to develop the next version (Phase II). In addition to changes in the material, phase II included a multi-headed microscope session. Interobserver agreement (kappa value) and level of agreement with the standard scorer (MBG) were determined. Kappa values between 0.21 and 0.40 are considered as fair agreement, between 0.41 and 0.60 as moderate agreement, and 0.61 and 0.80 as good agreement between observers.

**Results:** In Phase I, 12 pathologists were asked to score 30 of 45 cases. Kappa ranged from 0.15 - 0.80 (mean: 0.47, median: 0.47). The degree to which each observer correctly scored low, intermediate, and high risk cancers compared the standard is as follows: low risk 0-83%, (median 0.55) intermediate risk 20-100%, (median 0.73) and high risk 64-100% (median 0.78). In Phase II, 8 pathologists (4 involved in phase I, and 4 new observers) scored 12 cases. Kappa ranged from 0.40 - 0.92 (median: 0.74). For each risk category, the range of agreement between MBG was as follows: low risk 0-100%, (mean 0.83, median 1.0) intermediate risk 25-100%, (mean 0.66, median 0.75) and high risk 80-100% (mean 0.98, median 1.00).

**Conclusions:** The mean Kappa for phase II demonstrates good interobserver agreement. Recognition of high-risk carcinomas had the greatest reproducibility. We conclude that the risk scoring can be taught to other practicing pathologists. A multi-headed microscope session is a fundamental aspect of learning surgical pathology. Not surprisingly, the addition of this segment improved interobserver reproducibility, and accuracy when compared to MBG. 1. Brandwein-Gensler(2005) Am J Surg Pathol 29: 167-178.

### 1065 Histological Factors Associated with Lymph Node Metastasis in Thyroid Papillary Carcinoma in a Brazilian Institution

*FBC Cavalcanti, ES Mello, VAF Alves, MR Vianna, C Loreto, Y Menezes.* Laboratorio CICAP, Sao Paulo, Brazil.

**Background:** The aim of this study is to identify the histological factors' frequency associated with lymph node metastasis in thyroid papillary carcinoma in a single Brazilian institution.

**Design:** We retrospectively analyzed the data from 1161 thyroidal cancer, in a period between 2003 and 2007. 1014 (87.3%) were papillary carcinoma and 113 (11.1%) had lymph node metastasis. In these cases the age, gender, tumor size, extra thyroidal extension, multifocality and histological variant were assessed.

**Results:** From 113 metastatic papillary carcinoma, 27 (23.9%) were men and 86 (76,1%) were female. 34 (30.1%) were over 45 years old, 1 (0.9%) was under 15 and 78 (69%) were between 16 and 44 years old. The histological papillary carcinoma variants were tall cell 1 (0.9%), diffuse sclerosing 2 (1,7%), oncocytic 6 (5.9%) and classical 95 (84.5%). The tumor size under 1 cm (microcarcinoma) were 48 (42.5%) and above 1cm were 65 (57.5%). Multifocality was present in 73 (64.6%) and extra thyroidal extension was present in 75 (66.4%) and absent in 38 (33.6%). In this last particular group, 21.1% were follicular variant while this variant was only present in 1.3 % of the tumors with extra thyroidal extension. We divided the microcarcinoma in

two subgroups: under 0.5cm and between 0.6 and 1.0cm. The subgroup under 0.5cm had 14 cases (12.3%). 7 cases (50%) had extra thyroidal extension, while multifocality was present only in 5 cases (28%).

**Conclusions:** In this particular Brazilian institution, the majority of the patients with metastatic papillary carcinoma were female between 16 and 44 years old. The most frequent histological variant was the classical papillary carcinoma. The factors usually accepted in literature associated with poor prognosis were, in decreasing frequency, extra thyroidal extension, multifocality and tumor size. These factors were not observed in the same frequency in microcarcinomas under 0.5cm, that were predominantly single (72%) and presented no difference concerning extra thyroidal extension.

### 1066 Non-Keratinizing Squamous Cell Carcinoma of the Oropharynx: A Distinct Clinicopathologic Entity

*RD Chernock, SK El-Mofty, W Thorstad, C Parvin, JS Lewis, Jr.*. Washington University School of Medicine, St. Louis, MO.

**Background:** Human papillomavirus (HPV)-associated squamous cell carcinomas (SCC) of the oropharynx are biologically distinct. They tend to present at a younger age and be associated with better survival compared to HPV negative tumors, despite early metastatic spread. A distinct, non-keratinizing histologic phenotype, characterized by nests of oval to spindled cells with hyperchromatic nuclei, scant cytoplasm and indistinct cell borders, has also been noted. Yet, clinicopathologic correlation of the non-keratinizing phenotype with patient outcome is lacking.

**Design:** Cases of oropharyngeal SCC with 2 to 8 years of clinical follow-up were identified from radiation oncology databases at Barnes-Jewish Hospital from 1997 to 2004. Patients received either definitive or postoperative intensity-modulated radiation therapy (IMRT). Tumors were classified as keratinizing (KSCC), non-keratinizing (NK) or hybrid (HYB) SCC. The latter shows overlapping morphologic features. In-situ hybridization (ISH) for high-risk HPV subtypes and immunohistochemistry for p16, a frequently up-regulated protein in HPV-associated carcinomas, were performed. Statistical analysis was done by log-rank tests for the equality of survivor functions, Cox Proportional Hazards regression analysis and chi-square tests.

**Results:** A total of 118 cases of SCC with an average of 40 months of clinical follow up were reviewed. There were 29 KSCC, 55 NK and 34 HYB SCC. NK and HYB SCC presented at higher stage and were more likely to be surgically resected with post-operative IMRT than K SCC. Tissue was available for HPV-ISH and p16 staining in 89 cases. NK and HYB SCC were significantly more likely to be HPV and p16 positive compared to KSCC (Table 1). Both NK and HYB SCC correlated with better overall survival compared to KSCC (p=0.0002 and p=0.0105, respectively) with no difference in survival between NK and HYB SCC (p=0.5386). Adjusting for treatment regimen did not alter the association.

Table 1. HPV and p16 Positivity

| Histologic Type | HPV | p16 |
|---|---|---|
| KSCC | 2/25 (8%) | 9/25 (36%) |
| HYB | 10/22 (45.5%)* | 18/22 (81.8%)* |
| NK | 29/42 (69%)** | 42/42 (100%)** |

*p<0.005, **p<0.001

**Conclusions:** The non-keratinizing histologic type is a distinct clinicopathologic entity. It strongly predicts HPV-association and better patient outcome, which may be due to its increased radiosensitivity. Hybrid SCC appears to be related to NK SCC. It also tended to be HPV and p16 positive and was similarly associated with better survival.

### 1067 Evaluation of Sinonasal Undifferentiated Carcinomas for Therapeutic Targets – EGFR, c-kit, and Her2/neu

*RD Chernock, A Perry, J Pfeifer, JA Holden, JS Lewis, Jr.*. Washington University School of Medicine, St. Louis, MO; University of Utah School of Medicine, Salt Lake City, UT.

**Background:** Sinonasal undifferentiated carcinoma (SNUC) is a rare and aggressive malignancy of uncertain histogenesis with limited treatment options. C-kit, epidermal growth factor receptor (EGFR) and Her2/neu are cellular transmembrane receptors that are over-expressed in a variety of human tumors and have become important therapeutic targets in anti-cancer therapy. Furthermore, it has been shown that immunohistochemical expression of EGFR, Her2/neu or of c-kit, particularly with activating mutations in the c-kit gene, can predict tumor response to these targeted therapies.

**Design:** A study was undertaken to identify the presence of potential therapeutic targets in SNUC. A total of 11 cases were retrieved from the Pathology Department files. Formalin-fixed, paraffin-embedded tissue (PET) sections were stained with monoclonal antibodies to c-kit, EGFR and Her2/neu. Slides were reviewed independently by two study pathologists and graded as follows: 0 = no staining; 1+ = less than 5%; 2+ = 5-25%; 3+ = 25-50%; 4+ = 50-100%. Fluorescence in-situ hybridization (FISH) for c-kit and chromosome 4 was performed on PET sections to identify the presence of either gene amplification or aneuploidy. A search for activating mutations in c-kit exons 9 and 11, which are the most frequently mutated exons in GIST, was undertaken by high resolution DNA melting curve analysis on DNA isolated out of PET blocks.

**Results:** Nine of the 11 cases (81.8%) were diffusely (4+) positive for c-kit in a membranous pattern. Only 3/11 cases (27.3%) were positive for EGFR. None of the cases were positive for Her2/neu. Activating mutations were not detected by high resolution DNA melting curve analysis in any of the 8 amplifiable tumors nor were specific c-kit gene amplifications seen by FISH. However, polysomy of chromosome 4 was detected by FISH in 4 of 8 of the informative tumors.

**Conclusions:** Our findings suggest that c-kit is frequently expressed in SNUC. The overexpression of c-kit is likely due to aneuploidy as activating mutations and specific gene amplification were not detected. SNUC may still be responsive to therapies targeting c-kit activating mutations, but our findings make this unlikely.

### 1068 An Immunohistochemical Comparison of Chordoma with Chondrosarcoma, Myxopapillary Ependymoma, and Chordoid Meningioma

*HY Cho, M Lee, H Takei, J Dancer, JY Ro, QJ Zhai.* The Methodist Hospital, Houston, TX; Gachon University Gil Medical Center, Incheon, Korea; Chosun University Medical College, Kwangju, Korea.

**Background:** Distinction of chordoma from other tumors with chordoid morphology such as chondrosarcoma, myxopapillary ependymoma, and chordoid meningioma has important clinical implications, since chordoma has a notably poor clinical course. However, there is enough overlap in their morphologic appearances and immunohistochemical findings, generating considerable diagnostic difficulty. Therefore, the judicious use of a panel of selected immunostains is unquestionably helpful in diagnostically challenging cases.

**Design:** In order to find a panel of immunohistochemical markers that have the greatest potential for assisting in distinguishing between chordoma and other tumors with chordoid morphology, an immunohistochemical study for EMA, pan-cytokeratin (panCK), GFAP, S-100, D2-40, galectin-3, E-cadherin, β-catenin, NCAM, and CEA was performed on 14 chordomas, 7 chondrosarcomas, 9 myxopapillary ependymomas, and 4 chordoid meningiomas.

**Results:** The immunohistochemical staining results are shown in above table. All chondrosarcomas showed strong immunoreactivity for D2-40. In contrast, all chordomas were negative for D2-40. D2-40 is shown to be very sensitive and specific marker in distinguishing these two histologically similar tumors. Immunohistochemical typing using a panel of EMA, panCK, and GFAP has allowed the complete differentiation of all tumors examined in this study.

**Conclusions:** In this study, we demonstrated that D2-40 is a useful chondroid marker that is very helpful for differential diagnosis between chordoma and chondrosarcoma. The best three immunohistochemical markers useful for the evaluation of tumors with chordoid morphology were EMA, CK, and GFAP.

Results of immunohistochemical stain (%)

| IHC marker | Chordoma | Chondrosarcoma | MP ependymoma | Chordoid menigioma |
|---|---|---|---|---|
| D2-40 | 0 | 100 | 67 | 33 |
| EMA | 100 | 0 | 0 | 100 |
| panCK | 100 | 0 | 22 | 0 |
| GFAP | 0 | 0 | 100 | 0 |
| S-100 | 92 | 100 | 67 | 0 |
| gal-3 | 100 | 57 | 44 | 100 |
| NCAM | 92 | 29 | 100 | 0 |
| b-cat | 0 | 0 | 0 | 100 |
| E-cad | 8 | 0 | 0 | 33 |
| CEA | 0 | 0 | 33 | 0 |

### 1069 Molecular and Phenotypic Analysis of Undifferentiated Skull Base and Paranasal Sinuses Neoplasms: An Integrated Approach to Diagnosis and Classification

*B Cordes, MD Williams, D Bell, EY Hanna, AK El-Naggar.* Univeristy of Texas, Houston, TX; M.D. Anderson Cancer Center, Houston, TX.

**Background:** Undifferentiated malignant neoplasms of the paranasal sinuses and skull base regions comprise biologically diverse entities with overlapping morphologic features. Ancillary studies including immunohistochemistry and molecular testing continue to evolve with respect to tumor evaluation. We assessed primary undifferentiated tumors in these regions and tumors which may extend to these areas for current complimentary methods available for optimal classification.

**Design:** Fifty primary sinonasal tumors (24 archival and 26 matched fresh and archival) and 28 additional head and neck tumors for comparison, accessioned at the U.T., M.D. Anderson Cancer Center from 1991-2006 formed this study. An immunohistochemical panel of epithelial, mesenchymal, neuroectodermaland melanocytic markers were performed on tissue microarray sections constructed from formalin fixed paraffin-embedded tumor materials. RT-PCR analysis of mRNA for EWS-FLI1 and PAX-FKHR fusion transcripts and the hASH1 gene were performed primarily on fresh tissue specimens and for comparison on selected matched archival materials.

**Results:** Immunohistochemical analysis results were concordant with the phenotypic assessment and the initial diagnosis in 53 tumors and led to the reclassifications of three tumors and resolved an aberrant and overlapping expression of markers in one additional tumor. The EWS-FLI1 fusion gene transcript was detected in four (8.0%) of the 50 tumors and were all among the 10 confirmed Ewing sarcomas. PAX-FKHR fusion transcript was not detected in any of the tumors including rhabdomyosarcomas. The hASH1 gene transcript was identified in 11 of 49 (22.4%) tumors; (3/6) neuroblastomas, (4/4) neuroendocrine carcinomas, (1/7) basal cell, (1/7) undifferentiated carcinomas, (1/8) rhabdomyosarcomas, and (1/8) melanomas.

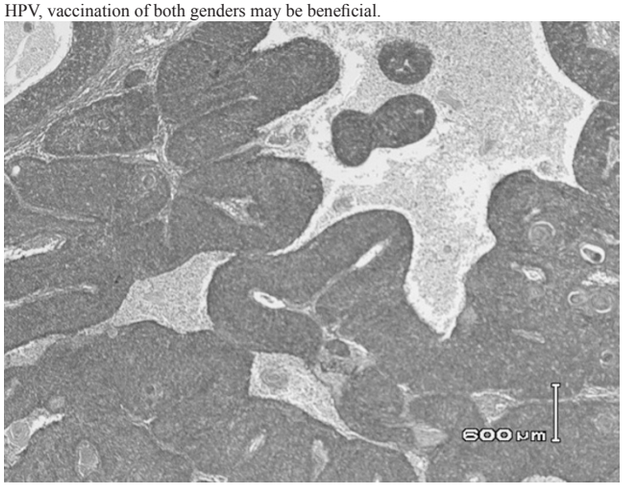
**Conclusions:** 1) An integrated approach to undifferentiated neoplasms of the paranasal sinus and skull base region is essential. 2) A panel of immunohistochemical markers allows for optimal classification in most cases avoiding potential problems with aberrant expression. 3) Molecular assessment for translocations may aid in confirming the diagnosis of undifferentiated neoplasms particularly for Ewing sarcoma/PNET. 4) hASH expression is not specific for olfactory neuroblastoma and is not recommended as part of the algorithm.

### 1070 Incidence of Human Papillomavirus in Head and Neck Squamous Cell Carcinomas

*PC Desai, P Gopal, MV Jaglal, G Schaefer, SJ Ghim, AB Jenson, HI Farghlay.* University of Louisville, Louisville, KY.

**Background:** Human Papilloma Virus (HPV) is found in up to 25-30% of head and neck squamous cell carcinomas (HNSCC) and has been shown to yield a better prognosis. However, currently there is no routine testing in HNSCC samples for HPV. The goal of this study is to expand our knowledge of the role of HPV in such lesions and evaluate

possible therapeutic implications.
**Design:** Surgical pathology records were searched for cases diagnosed as neck lymph nodes with metastatic squamous cell carcinoma from January 1, 2000 to July 31, 2007, which yielded 41 patients (7 female and 34 males) ranging in age from 38-81 years (mean 56 yrs). The sites of the primary squamous carcinomas included oral cavity, larynx, hypopharynx, oropharynx, and other unknown primaries. Cases of HNSCC were evaluated for the presence of HPV DNA by utilizing 23 mucosotropic HPV subtypes that included 6, 11, 16, 18, 33 and 45. Paraffin blocks were also stained for the p16INK biomarker.
**Results:** The overall HPV incidence rate was 12/41 (29%) by PCR. 11 samples were positive by both PCR and p16 staining, 1 was positive with only PCR, and 4 additional samples were positive with p16 staining only. HPV subtypes were determined by sequencing of the positive PCR product yielding 10/12 HPV 16, 1/12 HPV 33 and 1/12 HPV 45.
**Conclusions:** This alarming rate of HPV presence in this study is higher than previously documented in any other studies. Since HPV has been documented to be positive prognostic indicator in men, HPV staining is recommended on neck squamous carcinomas. Future directions of studies may include more in depth assessments of whether the HPV is simply present in these samples causing a predisposition to HNSCC or whether the HPV is integrated into the DNA and producing E6, E7 causing deactivation of tumor suppressing genes. Regardless, given the high incidence rate of HPV, vaccination of both genders may be beneficial.




### 1071 Pitfalls in the Diagnosis of Gnathic Osteosarcomas

*PI DeVilliers, MJ Klein, GP Siegal.* University of Alabama, Birmingham, AL.
**Background:** The histologic presentation of gnathic osteosarcomas, especially those that are not predominantly chondroblastic, can differ from the long bone variants, in that although presenting as a high grade tumor, they are associated with a lower potential for metastasis and have a better 5-year survival rate. Primary pediatric gnathic osteosarcomas (in patients 18 y/o or younger) are extremely rare and usually present as low to intermediate grade osteoblastic lesions. The presence of bone-forming pathologic processes unique to the jaws including osseous dysplasia, condensing osteitis, central ossifying fibroma and cementoblastoma makes the diagnosis of osteosarcoma more challenging. Central low grade osteosarcoma can mimic Paget's disease or fibrous dysplasia, while gnathic osteoblastoma can be a diagnostic dilemma in osteosarcoma. Furthermore, parosteal osteosarcoma can resemble peripheral ossifying fibroma. The radiographic features of gnathic osteosarcoma include a periosteal reaction, widening of the periodontal ligament space and structural changes in the mandibular canal along with the features typical of an aggressive, destructive process.
**Design:** We had the opportunity to review our experience over the last 2 decades at the University of Alabama at Birmingham with these neoplasms. The epidemiologic data as well as the radiographic and clinical features were extracted from the information recorded from the biopsy submission forms as well as from the radiographs on file; the slides were retrieved and examined by two bone pathologists and one oral pathologist.
**Results:** Our records showed the age of onset to be, on average, 20 years later than for osteosarcoma of long bones (peak age second decade), suggesting that the growth of the jaw has no relationship as an etiologic factor. Of interest, low grade central osteosarcoma showed a preference for the mandible and represented a diagnostic dilemma in one patient with McCune-Albright syndrome while parosteal osteosarcoma presented more commonly in the maxilla, arising from the alveolar ridge.
**Conclusions:** Correlation of clinical, histologic and radiographic findings will be emphasized, in order to explain how we are able to reach an accurate diagnosis and we will utilize selective cases from our archives to illustrate these diagnostic dilemmas.

### 1072 Pure Salivary Duct Carcinomas Can Be Classified into Luminal, HER2 and Basal-Like Phenotypes

*S Di Palma, A Skalova, M Ungari, A Sandison, H Peterse, C Marchio, JS Reis-Filho.* Royal Surrey County Hospital, Guildford, United Kingdom; Charles University, Plzen, Czech Republic; Universita degli Studi di Brescia, Brescia, Italy; Charing Cross Hospital, London, United Kingdom; NKI, Amsterdam, Netherlands; The Breakthrough Breast Cancer Research Centre, London, United Kingdom.
**Background:** Salivary duct carcinomas and invasive ductal carcinomas of the breast have remarkably similar morphological features. It has been recently demonstrated that invasive breast cancers can be subclassified into luminal, HER2 and basal-like cancers and that these groups have distinct molecular characteristics and clinical behaviour. The aim of this study was to apply an immunohistochemical panel previously validated for breast cancer to determine whether salivary duct carcinomas could also be classified into these molecular groups (i.e. luminal, HER2 and basal-like).
**Design:** 27 pure salivary duct carcinomas were retrieved from the files of the contributing authors. All cases were reviewed by one of the authors, histo-typed and graded according to the WHO classification. Representative sections were stained with antibodies for oestrogen receptor (ER), androgen receptor (AR), HER2, epidermal growth factor receptor (EGFR) and cytokeratin (Ck) 5/6. HER2 and EGFR were scored according to the Herceptest scoring system. Cases with 2+ or 3+ expression were considered positive. Cases were classified as of HER2 phenotype if they expressed HER2 3+, regardless of the expression of other markers; tumours that lacked HER2 expression and expressed either ER or AR were consider of 'luminal' phenotype, regardless of the expression of other markers. Cases that lacked HER2, ER and AR and expressed either EGFR or Ck 5/6 were considered of 'basal-like' phenotype. Cases that lacked all markers were considered of indeterminate phenotype.
**Results:** Positivity for ER, AR, HER2, EGFR and Ck 5/6 was seen in 0 (0%), 23 (85.2%), 4 (14.8%), 5 (18.5%) and 3 (11.1%) cases, respectively. Out of the 27 cases, 14.8% were of HER2 phenotype, 70.4% were of luminal phenotype, 3.7% were of basal-like phenotype and 11.1% were of indeterminate phenotype.
**Conclusions:** We demonstrate that in a way akin to breast cancer, salivary duct carcinomas can also be classified according to the molecular subgroups. Most importantly, we report for the first time the existence of a subgroup of pure salivary duct carcinomas that have the typical immunohistochemical profile of 'basal-like' carcinomas. These results may help understand the biological diversity of salivary duct carcinomas.

### 1073 Utility of Frozen Section Diagnosis in the Management of Invasive Fungal Sinusitis

*S El-Zayaty, A Langerman, JB Taxy.* The University of Chicago Medical Center, Chicago, IL.
**Background:** Invasive fungal sinusitis is a potentially life-threatening infection occurring in immunocompromised patients. Histologically, fungal elements exhibit extensive tissue and vascular invasion, often in the background of necrotic debris. Management of these patients involves aggressive surgical debridement in conjunction with systemic antifungal agents.
**Design:** The surgical pathology archives at the University of Chicago Medical Center were searched from 1995 to 2007. Ten cases of invasive fungal sinusitis were reviewed. Clinical records including results of microbiologic cultures and imaging studies were obtained and results subsequently correlated with the histologic findings. The long-term clinical outcome of all patients post-operatively to date was reviewed.
**Results:** All ten patients were severely immunocompromised, with varying underlying etiologies ranging from hematologic malignancies to diabetic ketoacidosis. Presenting symptoms generally included fever and orbital proptosis. The involvement was typically a bilateral pansinusitis. Radiographic progression of infection into the cranial vault was common. Perineural invasion was prominent, especially in Mucor cases. All but two patients required multiple debridements, with one requiring up to eleven procedures. Intraoperative frozen section was requested in all ten cases. Fungal organisms were readily identified on frozen section in every case by hematoxylin and eosin staining. A Diff-Kwik stain was also useful. The causative organism varied, but Aspergillus spp. predominated (6/10). The remaining cases included Mucor (2/10), Alterneria (1/10), and mixed fungal infections (1/10). In 8 of 10 cases the fungal genus was identified on frozen section and was confirmed by microbiology cultures. In the remaining two cases the frozen section identified organisms, but cultures were negative. Clinically, nine of ten patients died of complications related to invasive fungal sinusitis, most commonly cerebral invasion.
**Conclusions:** Intraoperative frozen section can be pivotal in the surgical management of invasive fungal sinusitis, and the fungal organisms can be readily identified on a standard hematoxylin and eosin stain. The organisms may vary and perineural spread is common. A viable surgical margin free of organisms is clinically sought, and often multiple debridements are necessary. Despite aggressive surgical management, most patients will succumb to invasive disease.

### 1074 Histopathologic and Clinical Features of Medullary Microcarcinoma and C-Cell Hyperplasia in Prophylactic Thyroidectomies: A Study of 42 Cases

*D Etit, WC Faquin, R Gaz, G Randolph, RA DeLellis, BZ Pilch.* Harvard University, Boston, MA; Ataturk Research and Training Hospital, Izmir, Turkey; Brown University Medical School, Providence, RI.
**Background:** Prophylactic thyroidectomies are increasingly being performed on patients at risk for the development of medullary thyroid carcinoma (MTC), and as a consequence, pathologists are more commonly encountering these specimens in routine practice.

**Design:** In this report, we present the detailed clinicopathologic features from a retrospective series of 42 prophylactic thyroidectomies for MTC that were performed based upon a family history of multiple endocrine neoplasia (MEN) or MTC, an elevated serum calcitonin level, or the detection of a RET proto-oncogene mutation.
**Results:** Patients included 22 men and 20 women with an age range of 2-73 years (mean age = 26.2 years). Among those with known RET proto-oncogene mutations, affected sites included exons 10, 11, 14, and 16. In 93% (N=39) of cases, either C-cell hyperplasia (CCH) (N=36) or medullary microcarcinoma, i.e. carcinomas <1.0 cm in diameter, (MMC) (N= 29) was found. CCH was often multifocal (N= 30) and bilateral (N= 23), and included both nonnodular and nodular patterns. Although calcitonin staining was used for confirmation, 94% of CCH cases were microscopically detectable using H&E stains. Some of the MMCs were round and well demarcated. Many MMCs were characterized histologically by the presence of a complex microarchitectural pattern with a desmoplastic stromal response (N= 29) and focal amyloid deposition (N= 12). Bilateral MMCs were present in 52% (N=15) of cases. The majority of MMCs exhibited a solid growth pattern (N=24). Three patients with MMC also had a concurrent incidental papillary thyroid microcarcinoma (PTC). Only one case of MMC showed evidence of metastatic disease to a pretracheal lymph node at the time of surgery. There was no evidence of recurrence among 29 patients where clinical follow-up was available (mean follow-up = 4.7 years).
**Conclusions:** Based upon the findings in this series, we conclude that despite their prophylactic nature, thyroidectomies in at-risk patients are very frequently associated with CCH and/or MMC; however, the clinical prognosis for these patients is good. Acknowledgement: This study partially supported by grant (B.02.1.TBT.0.06.01-219.01-880-5768) from The Scientific and Technological Research Council of Turkey (TUBITAK).

### 1075 Eosinophil-Rich Squamous Carcinoma of the Oral Cavity: A Study of 12 Cases and Delineation of a Possible New Entity

*G Falconieri, S Pizzolitto, G DeMaglio.* General University Hospital, Udine, Italy.
**Background:** An excess of tissue eosinophils is rarely observed in association with solid tumors including squamous cell carcinoma (SCC). Over the years, their significance has been reappraised from time to time, either as a potential diagnostic tool for the assessment of stromal invasion or as a prognostic indicator. However, only a few studies have appeared that focus on eosinophil-rich SCCs arising within the oral cavity.
**Design:** All our cases of SCC of the oral cavity with eosinophilic infiltrates were retrieved from departmental files. Pertinent medical charts and follow-up information were obtained. Microscopic evaluation was carried out on routinely processed tissues. Arbitrarily, it was decided that in order to be included in the present series, more than 100 eosinophils per high-power field (HPF: 40x objective; 20x eyepiece) for at least 10 consecutive HPFs must be present within tissue sections of the tumors.
**Results:** The case series included 12 patients (9 males and 3 females, age range 54 to 92 years, median 71 years). Tumors involved the gingival mucosa (5 cases), palate (2), tongue (2), palatine tonsil (2), and the mucosal aspect of the lip (1). Treatment modalities included radical surgery with homo- or bilateral neck dissection (6 cases), wide local resection (4), and palliative radiotherapy (2). Microscopically, all the tumors were infiltrating SCCs associated with abscess-like pools of eosinophils. The depth of tumor invasion ranged from 0.2 to 0.7 cm. In 2 cases the degree of eosinophil infiltrate obscured fine cytological details and made microscopic assessment of tumoral infiltration difficult. Lymph node metastases were documented in 4 patients and featured heavy tissue eosinophilia as well. Follow up ranged from 12 to 60 months (median 36). There was no evidence of progressing disease and no patient died of tumor.
**Conclusions:** Heavy tissue eosinophilia is invariably associated with SCC that has invaded the mucosal lamina propria, or beyond. An eosinophil-rich infiltrate may be a valuable microscopic finding in the initial assessment of oral cavity SCC especially when tumor invasion cannot be confidently established based on conventional criteria. Data from our series also suggest that the customary aggressiveness of oral cavity SCC may be mitigated by an excess of tissue eosinophils. Eosinophil-rich SCC may therefore segregate within a potential entity pursuing a more favorable course when compared to ordinary SCC.

### 1076 Immunoreactivity for p53 in Human Head and Neck Cancer May Change after In Vitro Cisplatin Exposure

*D Fanni, P Coni, L Pilloni, G Senes, G Faa.* University of Cagliari, Cagliari, Italy.
**Background:** p53 is a tumor supressor protein, which functions by halting cellular proliferation in response to a variety of cellular stresses. Specific stress signals result in post-translational modifications of p53 that induce activation of distinct transcriptional programmes and outcomes in the cells in order to inhibit cellular proliferation through cell cycle arrest, senescence or apoptosis. Loss of p53 function is a common feature of human cancers. Overexpression of p53 appears to be mainly due to the increased stability of the mutated p53. The inactive conformation of the wild type of p53 has been also reported as a possible cause of its overexpression. The aim of this study was to evaluate immunoreactivity for p53 in human head and neck cancer, before and after cisplatin exposure.
**Design:** Fourteen squamous cell carcinomas of head and neck, 3 from the oral cavity and 11 from larynx, were collected. Fresh samples were incubated in an inorganic medium, containing or not containing cisplatin (Teva L01XA01 at 75 µg/ml), for 30 minutes at 37°C and pH 7,4. A specimen from each tumor was immediately formalin-fixed. After incubation all specimens were formalin-fixed, paraffin-embedded and routinely processed. Immunohistochemical staining was performed using antibodies against p53 (Dako Denmark A/S, Glostrup, Denmark, clone DO-7).
**Results:** p53 overexpression was detected in 12 out of 14 tumor cells, when studied before cysplatin exposure. 4 out of these 12 tumour samples showed a considerable decrease in p53 immunoreactivity following cysplatin treatment; 3 were from larynx and 1 from the oral cavity. In the other 8 cases, 6 from larynx and 2 from the oral cavity, immunoreactivity for p53 did not change after cisplatin exposure. The last 2 tumors from the larynx did not show any reactivity for p53 after cysplatin.
**Conclusions:** Our preliminary data show an intertumoral striking variability in the ability of cysplatin to modify the p53 immunoreactivity in head an neck cancer. In 4 cases, we were able to normalize the expression of p53 in tumor cells, after 30 minutes of in vitro cisplatin exposure, suggesting that, in those patients, cisplatin could really act on tumor cells at molecular level. The absence of modifications in p53 status in the other 8 tumors, could indicate the inhability of cisplatin to interfer with the tumor cells. Further studies are required to confirm, at clinical level, our preliminary data, in order to establish if our simple and fast method could be useful in the choice of a specific chemotherapy.

### 1077 Spindle Epithelioid Cell Tumor with Thymus-Like Elements (SETTLE): A Morphological, Immunohistochemical and Molecular Genetic Study of 13 Putative Cases

*AL Folpe, RV Lloyd, CE Bacchi, J Rosai.* Mayo Clinic, Rochester, MN; Consultoria Patologia, Botucatu, Brazil; Centro Diagnostico Italiano, Milan, Italy.
**Background:** SETTLE is an extremely rare tumor of the thyroid and adjacent soft tissues. The histologic features of SETTLE overlap with those of synovial sarcoma (SS), and the distinction between these tumors may be extremely difficult on morphologic grounds. Very few cases of SETTLE have been studied for SS-specific SYT-SSX gene fusions. We performed a detailed morphologic, immunohistochemical and RT-PCR study of 13 cases in which the diagnosis of SETTLE was considered.
**Design:** All slides were reviewed and the morphologic features carefully recorded. Immunostains for low molecular weight cytokeratins (LMWCK), high molecular weight CK (HMWCK), EMA, CK7, CK20, CD34, CD117, CD99, BCL-2 and INI-1 were performed. Paraffin section RT-PCR for SYT-SSX1/SSX2 was performed using published techniques.
**Results:** The tumors arose in 8F and 5M, with a median age of 13.5 years and involved the thyroid in 12/13 cases. All tumors consisted predominantly of spindled cells with low to intermediate nuclear grade, arranged in infiltrative fascicles and vague nests. Stromal hyalinization, microcystic change and scattered small glands with mucin production were often seen. Two cases showed more extensive epithelial differentiation (up to 30%). Wiry collagen, numerous mast cells and intraglandular necrotic debris were present in 2 cases; calcifications were absent. Mitotic activity ranged from 1-15 MF/10HPF. Necrosis and vascular invasion were present in 1 case. IHC results were: LMWCK (9/10), HMWCK (8/9), EMA (7/10), CK7 (8/10), CK20 (0/10), CD34 (0/10), CD117 (5/9), CD99 (7/9), BCL-2 (9/10) and INI-1 (9/9). RT-PCR results were: positive (1/12), negative (8/12), non-diagnostic (3/12).
**Conclusions:** The distinction between SETTLE and SS on morphologic grounds is extremely challenging, as evidenced by our finding of at least 1 genetically confirmed SS within our series of putative SETTLE. The presence of extensive stromal hyalinization and microcystic change, and the absence of intraglandular necrotic debris, wiry collagen and numerous mast cells favor SETTLE. Although there is considerable immunophenotypic overlap between SETTLE and SS, uniform CK expression is only seen in SETTLE. RT-PCR for SS-associated fusion genes may be useful in morphologically and immunohistochemically indeterminate cases. Additional studies such as FISH may be helpful in clarifying the exact nature of indeterminate cases in which RT-PCR study is non-diagnostic.

### 1078 Array Comparitive Genomic Hybridization Analysis of Olfactory Neuroblastoma

*M Guled, S Myllykangas, HF Frierson, SE Mills, S Knuutila, EB Stelow.* Haartman Institue and HUSLAB, University of Helsinki, Helsinki, Finland; University of Virginia, Charlottesville, VA.
**Background:** Olfactory neuroblastomas (ONBs) are rare malignancies that arise in the sinonasal tract. Although some larger clinicopathologic studies have been published, little is understood regarding the molecular and cytogenetic abnormalities of the tumors. Furthermore, consistent and specific genetic changes have not been identified. This study used array-based comparative genomic hybridization (aCGH) to identify DNA copy number changes in ONBs.
**Design:** A single institution's surgical pathology database was searched for surgically resected ONBs. Cases were reviewed and 15 cases were selected based on the availability of abundant and well-preserved, paraffin-embedded neoplastic tissue. Khadish stage, Hyams grade and follow-up data were recorded for these cases. DNA was extracted from concentrated, microdissected tissue and hybridized with an equal amount of reference DNA to Agilent's 4x44 K CGH microarray (containing approximately 44,000 oligonucleatide probes). Hybridized arrays were scanned using an Agilent DNA microarray scanner and data were analyzed using Agilent CGH Analytics Software 3.4.
**Results:** Thirteen cases had analyzable results. There were slightly more DNA copy number gains than losses. With a threshold set to include only those alterations shared by at least 20% of the cases 21 regions showing gain were identified: 1p36.31, 1p35.3, 4p16.2-p16.3, 4p12-p15.31, 4q12, 4q21.22-q22.1, 4q27-q35.2, 5q34, 5q35.1-q35.3, 6p12.3, 7q11.22-q21.11, 9p13.3, 10p12.31, 12q23.1, 12q24.31, 13q, 15q13.3, 16q12.1, 20p/q, 21q and Xp/q. Twenty-one regions showing loss included: 2q31.1, 2q33.3, 2q37.1, 4p13, 5q31.2, 6p22.1, 6q21.33, 6p12.3, 6q16.3, 6q21, 6q22.1, 15q11.2, 18q12.2-q12.3, 19p12, 19q12, 19q13.11, 19q13.32, 19q13.43, 22q11.23, 22q12.1 and Xp/q. Higher stage tumors showed more alterations. Loss of Xp21.1 and gains of 13q14.2-q14.3, 13q31.1, and 20q11.21-q11.23 were present in 66% of stage 3 cases. Gain at 5q35, 13q, and 20q and losses at 2q31.1, 2q33.3 and 6q16-q22 were present in 50% of the cases.
**Conclusions:** This study has identified novel chromosomal regions which are frequently altered in ONB. Furthermore, the results indicate that 20q and 13q may be important in the progression of ONB. Further studies investigating specific oncogenes and tumor repressor genes throughout these sites may be warranted.

### 1079 Activated MAP-Kinases Show Coordinated Expression in Salivary Gland Adenoid Cystic Carcinoma

*A Handra-Luca, P Menard, P Fouret.* APHP GHU Nord, Bondy, France; APHP GHU Ouest, Paris, France; Institut de Cancerologie Gustave-Roussy, Université Paris 6, Villejuif, France.

**Background:** Little is known regarding the mitogen-activated protein kinase (MAPK) activation state in salivary gland adenoid cystic carcinoma (ACC).

**Design:** We evaluated expression of the phosphorylated forms of ERK (pERK), JNK (pJNK), and P38 (pP38), as well as that of Ki67 by immunohistochemistry in paraffin-embedded tumor specimen from 61 patients. The median value of the H-score (the proportion of stained tumour nuclei multiplied by the staining intensity of nuclei) and of the percentage of the Ki67 tumor nuclei were used as cutoff for classifying tumors as with high or low expression.

**Results:** The sex ratio was 15 men to 46 women. The median age was 50 years. Forty-two out of 61 tumors (69%) developed in the minor salivary glands. The median tumor size was 20 mm. The 23 cases with stage IV presented with bone infiltration (17), skin or facial nerve extension (3), or metastases to the lungs (3). Tumors were heterogeneous in their expression of pERK, pJNK, and pP38, which were expressed at high levels in 57%, 79% and 63% ACC, respectively. The rankings of scores for pairs of activated MAPK across tumors were correlated (Table).

Correlations between paires of activated MAPKs (Kendall rank coefficient)

| Pair | tau | p-value |
|---|---|---|
| pERK, pP38 | 0.38 | 0.0004 |
| pERK, pJNK | 0.33 | 0.002 |
| pP38, pJNK | 0.45 | <0.0001 |

The correlation was the strongest for pP38 and pJNK. The Ki67 index was high in 33/60 ACC. Among clinicopathologic characteristics, perineural invasion with high pJNK and high Ki67 index (p=0.005 and p=0.03, respectively). In the 42 patients with follow-up complete data for more than 5 years, pP38 (adjusted risk ratio 0.91, 95% CI 0.86 - 0.97) and Ki67 (adjusted risk ratio 1.05, 95% CI 1.00-1.10) could be used to fit a Cox regression model to overall survival (p=0.009). In univariate analysis, patients with pP38 positive tumors survived longer than those with pP38 negative tumors, but this was not significant.

**Conclusions:** This study provided evidence that the activated MAPK pERK, pJNK and pP38, were coordinately expressed in salivary gland adenoid cystic carcinomas. Perineural invasion was correlated to high Ki67 index and high pJNK expression. Overall survival was predicted by p38, independently of tumor cell proliferation.

### 1080 Hypocellular (Collagenized) Spindle Cell Squamous Carcinoma of the Head and Neck (SCSCHN): A Light Microscopic and Immunohistochemical (IHC) Study

*D Hoehn, BM Wenig.* St Lukes Roosevelt Hospital Ctr., New York, NY; Beth Israel Medical Ctr., New York, NY.

**Background:** SCSCHN is a high-grade variant of conventional squamous cell carcinoma (SCC) histologically characterized by the presence of differentiated SCC and an associated malignant spindle-shaped and pleomorphic cell infiltrate. Typically, the spindle-shaped and pleomorphic cell infiltrate is hypercellular and readily identified as malignant. In a majority of cases, cytokeratin immunoreactivity is present; however, in up to 40% of cases cytokeratin staining is absent. Further, differentiated SCC may be absent. SCSCHN occasionally appears hypocellular with prominent stromal collagenization. In the absence of differentiated SCC and cytokeratin staining, the diagnosis may be overlooked or misinterpreted as a reactive process. We report the clinical, pathologic and IHC findings in 3 cases of hypocellular SCCHN and detail the features allowing for its diagnosis.

**Design:** We identified 3 cases of hypocellular SCSCHN from our files. The clinical and light microscopic features were reviewed. IHC analysis was performed on paraffin sections using standard avidin-biotin complex method with microwave antigen retrieval for cytokeratins (CK), including AE1/AE3, CAM5.2, and CK907 (34bE12), as well as for p63 and p16.

**Results:** All 3 cases occurred in men ages 66, 70 and 73. Clinical complaints included hoarseness and dysphagia. The lesions were polypoid and were localized to the larynx (vocal cord) (n=2) and base of tongue (n=1). Histologic features included the presence relatively hypocellular infiltrate with prominent stromal hyalinization. Despite the paucicellular infiltrate, there was marked nuclear pleomorphism and increased mitotic activity, including atypical mitoses. Foci of differentiated SCC in the form of keratinizing dysplasia or invasive SCC were present in 2 cases; osseous metaplasia was found in 1 case. Immunoreactivity included AE1/AE3 (3/3), CAM5.2 (1/3), CK907 (3/3), p63 (3/3) and p16 (0/3).

**Conclusions:** Hypocellular (collagenized) SCSCHN may present diagnostic difficulties due to the relative dearth of cellularity usually associated with this tumor type. The diagnostic challenge may be magnified in limited biopsy sampling of such polypoid lesions. Awareness that a hypocellular variant of SCSCHN exists should alert the pathologist to such a possibility and attention to the morphologic details with or without IHC support will facilitate an accurate diagnosis.

### 1081 A Short, Cyclic RGDLASL Peptide Which Specifically Interacts with Integrin αvβ6 Can Be a Valuable Tool for Targeting All Major Kinds of Head and Neck Squamous Cell Carcinoma

*JR Hsiao, KF Hsu, YT Jin.* National Cheng Kung University, Tainan, Taiwan.

**Background:** Using a disulfide-constrained, cyclic (C7C) phage display library, we have selected a numer of candidate phage clones that can bind oral squamous cell carcinoma (OSCC) cells with high affinity. Phage clone-29, which contains a short, 7-mer cyclic RGDLASL peptide, is rapidly internalized by OSCC cells. Although it is reported that linear 20-mer peptides containing RGDLXXL sequence can interact with integrin αvβ6 specifically, whether this short, cyclic RGDLASL peptide retains this specificity is not known. We thus explored the receptor specificity for this cyclic peptide. Besides, potential usage of this peptide sequence for targeting all major kinds of head and neck squamous cell carcinoma (HNSCC), including OSCC, was also studied.

**Design:** Phage immunocytochmistry was used to study internalization of phage-29. Competition assay by cognate or scrambled peptides was used to confirm sequence-specificity of internalization. Interaction of phage-29 with αv-associated integrins was studied by fluorescence confocal microscopy. Phage immunohistochemistry was used to demonstrate the interaction of phage-29 particles with its receptors on human oral cancer specimens. Thirty-eight HNSCC specimens, including oral cavity (6), oropharynx (3), nasopharynx (14), larynx (4) and hypopharynx (11), were used to study the expression patterns of putative receptors of phage-29 by immunohistochemistry.

**Results:** Internalization of phage-29 by OSCC cells can be totally inhibited by cognate RGDLASL but not by scrambled RGELASL peptides, suggesting sequence-specificity. Using different cell lines which naturally express various kinds of surface αv-associated integrins, it is confirmed that phage-29 preferentially binds to αvβ6 without interacting with αvβ1, αvβ3 or αvβ5. On human oral cancer specimens, we clearly demonstrated that phage-29 particles can target oral cancer cells in an αvβ6-dependent manner, without interacting with normal oral epithelium. We also showed that, in addition to OSCC, integrin αvβ6 is expressed in all major kinds of HNSCC (94.7%, 36/38), further extending the clinical potential of this αvβ6-targeting peptide sequence.

**Conclusions:** A short, cyclic RGDLASL peptide selected from phage display library specifically interacts with integrin αvβ6. This αvβ6-interacting peptide can be a valuable tool for targeting all major kinds of HNSCC.

### 1082 *KIT* Mutations in Mucosal Melanoma

*E Jacobson-Dunlop, C Le, J Patterson, A Warrick, A Harlow, S Azar, F Cruz, A Town, BP Rubin, FS Hodi, S Muller, MC Heinrich, CL Corless.* Oregon Health & Science University, Portland, OR; Cleveland Clinic, Cleveland, OH; Dana-Farber Cancer Institute, Boston, MA; Emory University, Atlanta, GA.

**Background:** Recent studies suggest that *KIT* gene mutations are present in mucosal melanomas. While the KIT inhibitor imatinib is ineffective in the treatment of cutaneous melanoma, we recently observed a dramatic clinical response in a patient with a rectal melanoma harboring a *KIT* mutation. We therefore surveyed a series of mucosal and other melanoma subtypes for mutations in *KIT*, *BRAF* and *NRAS* to determine what fraction of tumors might be amenable to kinase inhibitor therapy.

**Design:** DNA from archival melanomas was amplified by PCR and the products were screened by denaturing HPLC for mutations in *KIT* exons 11, 13 & 17 (n=160), *BRAF* exon 15 (n=186), *NRAS* exon 1 (n=184) & 2 (n=84). Mutations were confirmed by direct sequencing. Immunohistochemistry for CD117 was performed on a subset of cases.

**Results:** *KIT* mutations were identified in 7/39 (18%) mucosal melanomas, including 2/4 rectal, 1/2 vulvar, 1 bladder, and 4/32 head & neck tumors. Mutations were less common in acral (1/7, 14.3%) and cutaneous (1/48, 2%) tumors, and absent in conjunctival (0/11) and uveal melanomas (0/56). Mutations included substitutions in exons 11 (W557R, V559A, L576P) or 17 (Y823D), and a duplication in exon 11 (PYDHKWE at E583). Notably, all of these mutations occur in GI stromal tumors and nearly all (except Y823D) are sensitive to imatinib. There were no mutations in cases additionally screened for *KIT* exons 8 (22 mucosal, 48 other) or 9 (4 mucosal, 103 other). CD117 expression was variable in *KIT* wild-type melanomas of all subtypes. Most, but not all, mutation-positive tumors had detectable CD117. The *BRAF* V600E substitution was present in cutaneous (18/61), conjunctival (4/16) and acral (2/5) melanomas, but not in mucosal (0/38) or uveal (0/66) tumors. *NRAS* exon 1 mutations were common in mucosal melanomas (7/37, 18.9%), rare in cutaneous (1/66), and absent in acral (0/5), uveal (0/61) and conjunctival (0/15) tumors; exon 2 mutations were limited to cutaneous melanomas (4/32). No tumor had more than 1 mutation.

**Conclusions:** Our findings confirm that *KIT* mutations of the type known to be sensitive to imatinib occur in 18% of mucosal and 14% of acral melanomas. *NRAS* exon 1 mutations are also common in these tumors, while *BRAF* mutations are absent. Genotyping can play an important role in the clinical management of these rare melanomas.

### 1083 IMP3, CD44, Ki-67, ß-1 Integrin, and Cyclin-D1 Expression in Radiation Resistant (RR) and Radiation Sensitive (RS) Laryngeal Squamous Cell Carcinomas (SCC)

*R Jaffar, RS Pieters, TJ Fitzgerald, DY Kim, SP Kadish, HT Jaffar, K Dresser, M Zona, ML Prasad.* UMASS Memorial Medical Center, Worcester, MA; Citizens Financial Group, Inc., Cranston, RI.

**Background:** Radiation therapy is frequently employed to treat head and neck SCC. While most tumors are radiation sensitive (RS), some fail to respond, and recur or continue to progress. Identification of radiation resistant (RR) tumors will allow their treatment with alternative or multimodality therapies. We studied various molecular markers in order to predict resistance to radiation.

**Design:** RS tumors (n=22) were defined as complete response/remission, and RR tumors (n=16) were defined as tumors with no or partial response with recurrence or progression over a 2 year follow-up period following radiation therapy. Tissue microarrays were constructed with pre-radiation biopsies and were immunostained with antibodies to insulin-like growth factor (IMP3), CD44, Ki-67, ß-1 Integrin, and Cyclin-D1. Expression in ≥30% of tumor cells was considered positive. Expression of IMP3 was assessed using a computerized image analyzer (Automated Cellular Imaging System). Expression was statistically correlated with available clinicopathological information e.g. radiation response, age [<60y (n=25), ≥60y (n=31)], gender (M 42, F 13), tumor size [T1&T2 (n=15), T3&T4 (n=12)], differentiation [well (n=19), moderate (n=24), poor/undifferentiated (n=10)], and location [supraglottic (n=20), glottic (n=24), combined (n=10)].

**Results:** IMP3 expression was significantly reduced in well-differentiated and glottic SCC. No correlation was found between the protein expressions and other clinicopathological parameters including radiation response.

Association of IMP3 expression with tumor differentiation and location

| **Differentiation** | **IMP3 Positive** | **IMP3 Negative** | **p-Value** |
|---|---|---|---|
| Well (19) | 1 | 18 | 0.0068 |
| Moderate (24) | 11 | 13 | |
| Poor and Undifferentiated (10) | 5 | 5 | |
| **Location** | | | |
| Supraglottic (20) | 12 | 8 | 0.0009 |
| Glottic (24) | 2 | 22 | |
| Combined (10) | 5 | 5 | |

**Conclusions:** Insulin-like growth factor (IMP3), adhesion molecules (CD44, ß-1 Integrin), proliferation marker (Ki-67), or cell cycle regulatory protein (Cyclin-D1) do not appear to be associated with radiation response. Reduced expression of IMP3 in well-differentiated and glottic SCC may suggest an association with better prognosis.

### 1084 A Reappraisal of Sinonasal Adenocarcinomas: Associations with Respiratory Epithelial Adenomatoid Hamartomas and Schneiderian Papillomas

*VY Jo, SE Mills, HP Cathro, EB Stelow.* University of Virginia, Charlottesville, VA.

**Background:** Sinonasal adenocarcinomas (SNACs) are rare. Those that do not resemble salivary gland tumors are classified as intestinal or non-intestinal and the latter are sub-classified as low-grade or high-grade. We have noted that some low-grade SNACs are associated with respiratory epithelial adenomatoid hamartomas (REAHs), also rare lesions that have recently been shown to be neoplastic. In this study, we review the morphology of non-intestinal SNACs with particular attention to potential precursor lesions, especially REAHs and Schneiderian papillomas (SPs).

**Design:** Our surgical pathology database was searched for all cases of SNAC diagnosed over a twenty-year period. All non-intestinal SNACs were examined. Tumors were designated as low-grade or high-grade, and low-grade SNACs were further classified as papillary, tubular or "other," based on the predominant growth pattern. Each case was also examined for concomitant REAHs or SPs.

**Results:** Thirty-six cases of non-intestinal SNACs were identified (16 women and 20 men; age range 14-89 years). Twenty-nine (81%) (15 women and 14 men; age range 39-89 years) were low-grade, and 7 (19%) (1 woman and 6 men; age range 32-78 years) were high-grade. High-grade lesions had predominately solid and trabecular growth with numerous mucocysts. Of these cases, 4 (57%) (4 men; ages 21, 22, 26 and 71) were associated with SPs. Of the low-grade lesions, 9 (31%) were papillary, 18 (62%) were tubular, and 2 (7%) were categorized as "other." Six (33%) low-grade tubular SNACs (2 women and 4 men; ages 43, 50, 59, 67, 67 and 68) were associated with REAHs. No other SNACs were associated with either REAHs or SPs.

Potential Precursors Associated with Sinonasal Adenocarcinomas

| Adenocarcinoma Type | Associated REAH | Associated Schneiderian Papilloma |
|---|---|---|
| Tubular | 6/18 | 0/18 |
| Papillary | 0/9 | 0/9 |
| Other | 0/2 | 0/2 |
| High Grade | 0/7 | 4/7 |

**Conclusions:** Non-intestinal SNACs are uncommon malignancies that can show a variety of growth patterns. Most of these are low-grade, frequently with either a tubular or papillary grow pattern. A subset, however, are high-grade, and demonstrate a distinct solid and trabecular growth pattern with intermixed mucocysts. Interestingly, some low-grade tubular SNACs are associated with REAHs while some high-grade SNACs are associated with SPs. Given the rarity of these various lesions, their coexistence suggests that both REAHs and SPs may be precursors of some SNACs.

### 1085 Prevalence of Human Papilloma Virus DNA in Sinonasal Undifferentiated Carcinoma

*B Joos, A Martin, M Nowacki, P Desai, A Jenson, H Farghaly.* University of Louisville, Louisville, KY; Norton Healthcare, Louisville, KY.

**Background:** Sinonasal undifferentiated carcinoma (SNUC) is a rare and highly aggressive malignant neoplasm of the nasal cavity and paranasal sinuses. The goal of this study is to evaluate the possible detection of human papilloma virus DNA (HPV) in SNUC and its possible prognostic importance, which to our knowledge, has not previously been reported.

**Design:** Surgical pathology records were searched for cases diagnosed as SNUC from January 1, 2000 through August 1, 2007, which yielded five patients (1 female and 4 males) ranging in age from 26-75 years (mean 56.8 yrs). The SNUC cases were compared to poorly differentiated squamous cell carcinomas (PDSCC) of the nasal cavity and paranasal sinuses from five patients (5 males), ranging in age from 53-75 years (mean 62.2 yrs). Immunohistochemical stains (IHCS) were performed for the following markers: EBV, CK 5/6, CK 7, CK14, CK19, EMA, NSE, chromogranin, p53, p63, S-100, HMB-45, desmin, MSA and LCA. IHCS were evaluated based on the percentage of tumor cells staining: <5%=negative, 5-50%=focally positive, 51-85%= positive, >85%= diffusely positive. Additionally, cases of SNUC and PDSCC were evaluated for expression of p16 and presence of HPV DNA utilizing 23 HPV subtypes that included 6, 11, 16, 18, 33 and 45. Touchdown PCR, a more sensitive of detection of HPV, was performed to verify the classical PCR results.

**Results:** The diagnosis of SNUC was confirmed in all cases by histology and IHCS. There was diffuse positivity of p16 in all SNUC cases. 2 of 5 PDSCC controls were positive for p16. HPV DNA was not detected in any of SNUC cases and was detected in 1 of 5 cases of PDSCC controls.

**Conclusions:** Although all SNUC cases demonstrated diffuse positivity of p16, this study demonstrates the absence of HPV in SNUC neoplasms.

### 1086 Proposing Diagnostic Criteria for Basaloid Squamous Cell Carcinoma of Head and Neck – An Entity Underdiagnosed on Biopsy

*SV Kane, S George, S Menon.* Tata Memorial Hospital, Mumbai, Maharashtra, India.

**Background:** Basaloid squamous cell carcinoma (BSCC), is a histologically distinctive & aggressive variant of SCC. There is a considerable morphological overlap between BSCC, adenoid cystic carcinoma (ACC) & poorly differentiated SCC (PDSCC). However the varied biological behavior & management necessitates their distinction on pretherapy biopsy. A confident diagnosis of BSCC in limited material is a difficult task as squamous differentiation or In-Situ carcinoma is often not seen.

**Design:** Total 130 cases of Head & neck BSCC [ biopsy and resection specimens ]diagnosed during a five year period were retrieved for study. Forty cases each of PDSCC & ACC [ resection specimens ] were selected for a comparative analysis of 18 standard histo-morphological parameters like nesting, basaloid cells, high nucleo-cytoplasmic ratio etc.An immunohistochemistry (IHC) panel comprising HMWCK, p63, MIB-1,CEA, calponin and c-kit was performed to distinguish BSCC from ACC. Data regarding clinical course, site of metastasis & treatment was collected. Standard statistical tests were applied using SPSS software. Based on the statistical analysis of histo-morphological features & immunoprofile of resected specimens,a set of diagnostic criteria for BSCC was proposed.

**Results:** Statistically significant histo-morphological parameters included specific tumour patterns, basaloid cells, comedonecrosis, increased mitosis, apoptosis, marked nuclear atypia with polypoidal nuclei and focal squamous differentiation. BSCC could be distinguished from PDSCC by patterns & basaloid cells. BSCC could be differentiated easily from ACC when squamous differentiation was noted. In absence of squamous differentiation which was a common occurrence in biopsies[60%], BSCC could be comfortably diagnosed with the aid of IHC. Diffuse & strong expression of p63 and HMWCK, high MIB-1 index, and negative expression of c-kit, calponin & CEA facilitated the distinction between BSCC and ACC.

**Conclusions:** Distinction of BSCC from ACC is difficult on biopsy in absence of squamous differentiation. A set of major & minor diagnostic criteria including morphologic and IHC parameters is proposed for diagnosis of BSCC on biopsy which enables proper therapeutic planning in Head & Neck cancer.

### 1087 Prognostic Factors of Recurrence in Carcinoma Ex Pleomorphic Adenoma (CXPA) with Emphasis on the Carcinoma Histologic Subtype: A Clinicopathologic Study of 34 Cases

*N Katabi, DL Carlson, D Gomez, N Lee, D Klimstra, R Ghossein.* MSKCC, NY, NY.

**Background:** CXPA are rare salivary gland neoplasms. There is a need to better stratify patients with CXPA, especially those with intra-capsular (IC)/minimally invasive (MI) tumors. Our aim was to identify pathologic predictors of outcome in CXPA, especially in the IC/MI category in order to better guide therapy.

**Design:** 34 cases of CXPA were identified over a 27 year period (1980-2007) and subjected to a detailed histopathologic analysis. IC/MI carcinomas were defined as tumors that were confined to or extended ≤1.5 mm beyond the capsule of the host PA.

**Results:** There were 12 IC/MI and 22 widely invasive (WI) tumors. There were 12 myoepithelial carcinomas (MC),19 salivary duct carcinoma, 2 adenocarcinoma N.O.S and 1 carcinosarcoma. The entire tumor size was similar between IC/MI and WI carcinomas (mean:3.5 and 3.6 cm respectively). There was a trend toward a higher frequency of MC in WI tumors (11/22, 50%) than IC/MI (2/12, 17%) CXPA (p=0.074). A similar trend towards a higher percentage of high grade tumors in WI (86%) versus IC/MI (58%) tumors was also found (p=0.097). WI carcinomas were mitotically more active than the IC/MI tumors (p=0.04). The PA was the major component in 4 of 8 (50%) IC/MI but in only 2 of 22 (9%) WI tumors (p=0.0009). Adequate follow up (FU) was available on 29 patients (median FU: 30 and 24 months for IC/MI and WI respectively). Three (2 IC, 1MI) of the 12 IC/MI tumors recurred while 10 of the 17 WI relapsed. Distant metatases occurred in 3 (25%) of the 12 IC/MI tumors and in 8 (47%) of 17 of the WI. Disease was present at last follow up in 1 (9%) of 11 IC/MI carcinomas and in 9 (53%) of 17 WI tumors (p=0.04). One (a MI tumor) of the 11 IC/MI (9%) and 7 of the 17 WI (41%) carcinomas caused death of the patients. There was no correlation between the grade of the carcinomas and recurrence. The recurrence rate was much higher in tumors containing MC (9/12, 75%) than in other types of carcinomas (4/17, 23.5%) (p=0.009).There was a significant correlation between the presence of MC and relapse in the IC/MI category in contrast to the WI tumors.Within IC/MI CXPA, all MC (2/2, 100%) recurred while only one (10%) of 10 non-MC relapsed (p=0.045).

**Conclusions:** 1) IC/MI CXPA can recur and cause death. 2) The presence of MC significantly increases the risk of recurrence in CXPA, even within the group of IC/MI tumors.

### 1088 Traumatic Ulcerative Granuloma with Stromal Eosinophilia: Reactive Ulcer or Lymphoproliferative Disorder?

*CL Kossover, SD Budnick, S Li.* Emory University, Atlanta, GA.

**Background:** Traumatic ulcerative granuloma with stromal eosinophilia (TUGSE) is a typically self-limited lesion of the oral mucosa with an unclear pathogenesis. Microscopically, it is characterized by a deep, dense, eosinophil-rich cellular infiltrate with scattered large atypical mononuclear cells. Recently, a few case reports have demonstrated CD30 expression on the large atypical cells and molecular evidence of T-cell clonality, suggesting that a subset of TUGSE could be the oral counterpart of CD30+ primary cutaneous lymphoproliferative disorders (LPDs). To our knowledge, however, a large study examining the presence of T cell clonality and CD30 expression in TUGSE has not been conducted.

**Design:** To study the clinical and histopathologic features and to examine the presence of T-cell monoclonality and CD30 expression in oral mucosal lesions with features of TUGSE.

**Results:** We selected 11 cases of TUGSE from the files of the Department of Pathology at Emory University. All cases presented as solitary lesions of the oral mucosa with a duration of 5 days to >6 months. Histologic examination of the tissue demonstrated ulcerative lesions with a dense cellular infiltrate extending deep between the muscle fibers and consisting of a variable number of inflammatory cells, including a predominant eosinophil population and scattered large atypical mononuclear cells. Immunohistochemical stains demonstrated rare to focal CD30 positivity on the large mononuclear cells in 7 of 11 cases. Polymerase chain reaction (PCR) studies demonstrated clonal T-cell receptor-γ gene rearrangements in 2 of the 11 cases, indicating that these two cases represented atypical T-cell LPDs. Of these 2 cases, only one demonstrated focal CD30-positive atypical mononuclear cells.
**Conclusions:** CD30-positive atypical mononuclear cells are present in cases of TUGSE lacking molecular evidence of T-cell clonality. Alternatively, cases of TUGSE with molecular evidence of T-cell clonality may not contain CD30-positive large atypical mononuclear cells. These results dispute the suggestion that a subset of TUGSE may be the oral counterpart of CD30+ primary cutaneous LPDs. Given that T-cell clonality was found in only 2 of the 11 cases studied, additional cases from our archives will be investigated in order to further explore the nature and classification of these atypical T-cell LPDs.

### 1089 Loss of or Reduced Expression of the von Hipple-Lindau Gene Product (pVHL) in Malignant Salivary Epithelial Neoplasms – With an Implication of Its Role in Tumorigenesis

*H Liu, J Shi, K Liang, S Meschter, F Lin.* Geisinger Medical Center, Danville, PA.
**Background:** The von Hipple-Lindau gene product (pVHL) has been linked to the carcinogenesis of both hereditary and sporadic clear cell renal cell carcinomas. Our recent study demonstrated that loss of expression of pVHL was observed in pancreatic intraepithelial neoplasia (PanIN) and invasive ductal adenocarcinoma of the pancreas (Lin et al. AJSP 2007; in press). In this study, we investigate the expression of pVHL in common salivary gland tumors.
**Design:** We immunohistochemically evaluated the expression of pVHL on conventional tissue sections in benign and malignant salivary gland tumors. A total of 61 cases was included in the study: group #1 (G1) – 4 cases of benign mixed tumor; group #2 (G2) – 3 cases of oncocytoma; group #3 (G3) – 24 cases of Warthin's tumor; group 4 (G4) – 22 cases of mucoepidermoid carcinoma; group #5 (G5) – 2 cases of adenoid cystic carcinoma; group #6 (G6) – 4 cases of acinar cell carcinoma; and group #7 (G7) – 2 cases of salivary duct carcinoma. The majority of cases also contained normal salivary gland tissue. The staining intensity was graded as weak or strong. The distribution was recorded as negative (less than 5% tumor cells stained), 1+ (5-25% of tumor cells stained), 2+ (26-50% of tumor cells stained), 3+ (51-75% of tumor cells stained), or 4+ (more than 75% of tumor cells stained).
**Results:** The results demonstrated a membranous and cytoplasmic staining pattern of pVHL in 24 of 24 cases (100%) in G3, with a diffuse staining (3+ or 4+) in 20 cases (83%); in contrast, only 5 of 22 cases (23%) in G4 were positive for pVHL, with diffuse staining in one case only. Diffuse and strong staining was also seen in normal salivary gland ducts, 3 of 3 cases in oncocytomas (G2), and ductal epithelial components in G1; whereas normal acinar cells and tumor cells from G5, G6 and G7 were all negative for pVHL.
**Conclusions:** The results demonstrate 1) that lost or reduced expression of pVHL is a frequent finding in malignant salivary gland neoplasms, suggesting that pVHL may play a role in tumorigenesis; 2) that pVHL is a useful marker in the distinction between acinar cell carcinoma from oncocytoma and in differentiating Warthin's tumor from mucoepidermoid carcinoma; and 3) that pVHL may potentially be used as a diagnostic marker in identifying malignant salivary gland neoplasms in small biopsy specimens or fine needle aspiration biopsy samples. Further study is warranted to confirm the above findings.

### 1090 High Levels of Expression of Cytokeratin 5 Is Strongly Correlated to Poor Survival in Mucoepidermoid Carcinoma

*NE Lueck, RA Robinson.* University of Iowa Hospitals and Clinics, Iowa City, IA.
**Background:** Mucoepidermoid carcinoma (MEC) is the most common malignant primary salivary gland tumor in adults. Little is known about cytokeratin expression in MECs or how it may relate to survival. In other organs, cytokeratin 5 (CK5) expression has been linked to a more aggressive tumor. In this study, we sought to determine the extent of CK5 expression in grade 2 and 3 MEC and correlate expression to survival. We also sought to compare CK5 to a number of oncogenic proteins that have been shown to play a role in the development of some MECs – Rb, p16 and p53 – as potential predictors of clinical outcome.
**Design:** Data on clinicopathologic features including stage and survival outcome were collected on 29 patients with grade 2 or grade 3 MECs which had followup for at least 4 years or until death. Staining with antibodies to CK5 protein, p16, Rb and p53 was carried out on paraffin-embedded tissue. Nuclear staining for p16 and Rb were considered positive and p53 overexpressed if greater than 10% of tumor cells were stained. Percent of tumor cells with cytoplasmic staining for CK5 was categorized as 0, 1(up to 25% cells staining), 2 (25-75%), or 3 (>75%). Fisher's exact test was used for evaluation of categorical variables and log rank analysis was used to assess equality of survival functions.
**Results:** 6 patients had grade 2 tumors and 23 had grade 3 tumors. The median patient followup was 33 months (range, 4 to 203) and 11 patients were alive at the end of the study. 5 patients had stage 1 or 2 disease (1 dead) and 24 had stage 3 or 4 disease (17 dead) and lower stage correlated to better survival ($P = .05$). 13/29 (44.8%) cases were positive for Rb, 10/29 (34.5%) positive for p16 and 5/29 (17%) overexpressed p53. No correlation existed for survival and expression of Rb or p16 or overexpression of p53 (log-rank test, $P = .73$, .79 and .71, respectively). 12/13 patients with highest (>75%) CK5 expression were dead while 6 of 18 patients with <75% expression were dead ($P = .006$, Fisher exact). When compared with those patients whose tumors had < 75% expression, patients whose tumors had the highest level of CK5 expression had much poorer survival times (log-rank test; $P = <.001$).
**Conclusions:** In this study p16, Rb and p53 did not predict survival in grades 2 and 3 MEC. There was a significant correlation between high CK5 expression and poor outcome. This suggests CK5 may be a potential marker for worse outcome in grade 2 and 3 MEC and additional studies are warranted.

### 1091 Sinonasal Tract Melanoma or Clear Cell Sarcoma? Molecular Evaluation of 4 Cases Diagnosed as Malignant Melanoma

*PL Lyle, CM Amato, JE Fitzpatrick, WA Robinson.* Penrose Hospital, Colorado Springs, CO; University of Colorado, Aurora, CO.
**Background:** Clear cell sarcoma (CCS) is a rare tumor typically associated with the tendons and aponeuroses of the distal extremities of young adults. CCS and malignant melanoma (MM) share immunohistochemical profiles and ultrastructural features, but classic CCS has characteristic morphology with low mitotic activity and minimal pleomorphism. Occasional cases, particularly those from the gastrointestinal tract, show pleomorphism, high mitotic index, and/or melanin pigmentation, making CCS indistinguishable from MM based on morphology. However, CCS is genetically distinct due to its consistent association with a t(12;22)(q13;q12) chromosomal translocation leading to the formation of the *EWS/ATF1* fusion transcript. This translocation has never been documented in cutaneous MM, and thus is regarded as specific for CCS. Recent evidence suggests that some primary "malignant melanomas" in unusual anatomic sites, most notably in the gastrointestinal (GI) tract, are actually CCS and may require molecular techniques for diagnosis. Primary sinonasal tract mucosal MM are rare tumors of "unusual location" that account for between 0.3% and 2% of all MM.
**Design:** Our study group consisted of 4 cases diagnosed as sinonasal tract MM in patients without a history of cutaneous MM. We utilized reverse-transcriptase polymerase chain reaction (RT-PCR) and fluorescence in situ hybridization (FISH) (dual-color breakapart probe) on formalin-fixed paraffin-embedded tissue to examine whether a proportion of cases diagnosed as sinonasal tract MM actually represent clear cell sarcoma.
**Results:** None of the four study cases harbored the *EWS/ATF1* fusion transcript by RT-PCR or FISH.
**Conclusions:** Although our study group is small, the findings suggest that sinonasal tract tumors with morphologic and immunohistochemical features of MM do not represent CCS based on molecular findings, and thus, are correctly diagnosed as MM.

### 1092 Investigation of Human Papillomavirus (HPV) Status by PCR, *In Situ* Hybridization, and $p16^{INK4a}$ Immunohistochemistry Supports an Etiological Role for HPV in the Majority of Tonsillar Carcinomas

*AL Matthews, WE Trotman, CS-C Adamson, MF Evans, K Cooper.* University of Vermont, Burlington, VT.
**Background:** Human papillomavirus (HPV) is the major etiologic factor in cervical cancer and is associated with many head and neck carcinomas, especially squamous cell carcinoma (SCC) of the tonsils. The HPV detection rate in tonsillar SCC ranges from 25-100% and most cases are caused by HPV-16. HPV has also been demonstrated in a subset of squamous papillomas (SP) of the tonsil. Expression of p16 protein represents a surrogate marker of HPV infection and biological activity in cervical cancers and may have a similar application in tonsillar carcinomas.
**Design:** Specimens of tonsillar SCC, corresponding lymph nodes (LNs) with metastatic SCC, and cases of SP (2000-mid 2006) were retrieved from departmental archives. DNA extracts from formalin-fixed, paraffin-embedded (FFPE) tissue blocks were tested for HPV using the GP5+/6+ PCR assay and dot-blot hybridization analysis for 37 HPV types. Tyramide-based chomogenic *in situ* hybridization (CISH) and $p16^{INK4a}$ immunohistochemical (IHC) staining (Clone JC8, LabVision) were then performed to corroborate PCR HPV typing data.
**Results:** A total of 85 specimens from 57 patients were assessed for HPV. Among 44 SCC/LNs, 38 (86.4%) were HPV positive by PCR, with HPV-16 (84.2%) and HPV-52 (7.9%) most prevalent. SCCs with basaloid features were positive (3/3) for HPV-16. Of 38 SCC/LN samples HPV positive by PCR, HPV was detected by CISH in 33 (86.8%; 32 punctate signals, 1 diffuse). Staining for p16 revealed that 28/31 (90.3%) SCC/LN samples HPV positive by PCR were also diffusely p16 positive, and 1/5 (20%) SCC/LN samples HPV negative by PCR was p16 positive. Nine of 24 (37.5%) SPs were HPV positive by PCR, and types included HPV-16 (77.8%) and HPV-6 and 11 (11.1% each). Of 8 SP samples HPV positive by PCR, HPV was confirmed by CISH in 1 (12.5%) instance (diffuse signals). None of the 22 (0/22) SPs were p16 positive.
**Conclusions:** There is a high prevalence of high-risk HPV types in tonsillar SCC and a close correlation between HPV positivity by PCR, punctate CISH pattern (indicating integrated HPV DNA), and diffuse p16 staining. These findings indicate that HPV plays a significant etiologic role in tonsillar carcinoma and that HPV vaccination programs may prevent a significant subset of these tumors. Conversely, the lack of correlation between PCR, CISH, and p16 data among SPs, with the lower HPV prevalence, suggests HPV may have a reduced role in tonsillar papillomas.

### 1093 Clinicopathologic Series of 44 Surgically Treated Sino-Orbital Osteomas with Emphasis on Cases with Osteoblastoma-Like Features

*JB McHugh, MM Yousef, B Sabb, DR Lucas.* University of Michigan, Ann Arbor.
**Background:** Symptomatic osteomas are uncommon benign tumors occurring almost exclusively in the head and neck. They occur most often in the paranasal sinuses with frontal affected most often followed by ethmoid, maxillary and sphenoid sinuses. Osteomas rarely recur after complete excision. Histologically, they can be divided into ivory and mature types, however, this separation is not thought to be clinically relevant. Osteomas may have osteoblastoma-like features (OBL) and some believe these behave more aggressively.

**Design:** 44 sino-orbital osteomas were identified between March 1986 and April 2007. Slides were reviewed and tumors categorized as ivory and/or mature and whether osteoblastoma-like features and Paget-like bone were present. We correlated these findings with clinical presentation and follow-up emphasizing those cases with OBL.
**Results:** Overall males outnumbered females (1.4:1) and mean age was 37 (range 12-70). 36% of sino-orbital osteomas had OBL. In the OBL group, males outnumbered females (3:1 vs 1:1) and mean age was similar (34 vs 40). All were symptomatic with headache, sinusitis, visual change, pain and proptosis being most common (at least one in 98%) and were similar between the two groups. Frontal sinus was most affected in both (64%) followed by ethmoid and maxillary with two orbital cases. Osteomas with OBL affected ethmoid more frequently and non-OBLoccurred more often in the maxillary sinus and orbit. OBL tumors more often extended into adjacent structures most often the orbit (38 vs 18%). Histologic subtypes and presence of Paget-like bone were similar between the two. Follow-up was available in 75% of OBL and non-OBL with medians of 21 (range 11-116) and 36 months (4-180). 67% of OBL and 81% of non-OBL were alive without disease and none died from disease. Non-OBL more often recurred (29 vs 8%) but less were alive with persistent disease (19 vs 33%) compared to OBL.
**Conclusions:** Sino-orbital osteoma pathology specimens are uncommon (average 4 cases/year) at our institution. Osteomas with OBL are not infrequent (36%), show a skew towards males and more frequently involve the ethmoid sinus than non-OBL. While the clinical features and outcomes were similar to the non-OBL group, they were more likely to show extracavitary extension. The significance of finding OBL does not appear to correlate with prognosis as some suggest and presence of OBL likely represents a spectrum of maturation versus a distinct clinicopathologic entity.

### 1094 miR-21 Upregulation and Loss of PTEN Expression in Head & Neck Squamous Cell Carcinoma

*MS McLemore, GJ Childs, NF Schlecht, TJ Belbin, JM Albanese, MB Prystowsky, M Brandwein-Gensler.* Montefiore Medical Center, Albert Einstein College of Medicine, Bronx, NY; Albert Einstein College of Medicine, Bronx, NY.
**Background:** MicroRNAs (miRNAs) are small RNA molecules that profoundly effect protein expression in human tissue by destabilizing mRNA or inhibiting its translation. Studies have linked miRNA expression to carcinogenesis. Many tumors (ie, colon) have been profiled for miRNA levels and show significant differences when compared to corresponding non-tumor tissue. To date, no study has characterized squamous cell carcinoma of the head and neck (HNSCC) for its unique miRNA signature.
**Design:** A custom printed microarray platform for miRNA was developed to evaluate miRNA expression in a cohort of 20 primary HNSCCs with matched mucosal samples (n=20). miRNA results were confirmed by RT-PCR. A validated target for miRNA-21 (miR-21) is mRNA encoding the PTEN tumor suppressor protein. PTEN expression was assessed using immunohistochemical (IHC) analysis (anti-PTEN at 1:200, Santa Cruz Biotech, Santa Cruz, CA) on tissue microarrays constructed from pathology specimens of this cohort and other patients with HNSCC (n=90).
**Results:** Many miRNAs were downregulated; in contrast, miR-21 was overexpressed in 13/20 cohort cases, compared to corresponding mucosa. PTEN expression was absent in 75% of all HNSCC cases. Due to core dropout, IHC data was available for 15/20 cohort cases (see Table).

miR-21 & PTEN Expression among HNSCC Cases

| Case # | miR-21 Expression (miR-21)* | Nuclear PTEN Expression (N)* | Cytoplasmic PTEN Expression (C)* |
|---|---|---|---|
| 1 | 0 | 0 | 0 |
| 2 | 0 | 0 | 0 |
| 3 | 0 | 1 | 2 |
| 4 | 0 | 0 | 0 |
| 5 | 0 | 0 | 0 |
| 6 | 1 | 0 | 3 |
| 7 | 1 | 0 | 0 |
| 8 | 1 | 0 | 3 |
| 9 | 1 | 0 | 0 |
| 10 | 1 | 2 | 2 |
| 11 | 1 | 0 | 1 |
| 12 | 2 | 0 | 0 |
| 13 | 3 | 0 | 0 |
| 14 | 3 | 1 | 1 |
| 15 | 3 | 0 | 0 |

*expressed as log ratio of tumor to mucosa concentration

PTEN was absent in 9/15 cases, including 5/10 with miR-21 upregulation (miR-21=1+). In 4/15 cases, both PTEN and miR-21 were lost.
**Conclusions:** Previous reports have indicated that loss of heterozygosity (LOH) and mutation in the PTEN gene locus cannot explain the frequency of PTEN loss in HNSCC. We confirm that PTEN is commonly absent in primary HNSCC. We demonstrate that miR-21 upregulation is another mechanism for PTEN loss; no tumors showed highly overexpressed miR-21 (miR-21=2+) with strong PTEN expression (N or C=2+). Loss of PTEN in the absence of miR-21 upregulation is likely due to other mechanisms, including LOH and DNA methylation.

### 1095 Bisphosphonate-Related Osteonecrosis of the Mandible Shares Similar Histologic Features with Osteoradionecrosis

*KT Montone, DC Stanton, VA LiVolsi.* University of Pennsylvania Medical Center, Philadelphia, PA.
**Background:** In patients with osteoporosis, bony metastases and multiple myeloma who have been treated with bisphosphonates (zoledronic acid (Zometa), pamidronate (Aredia), ibandronate (Boniva), etc., the phenomenon of bisphosphonate-related osteonecrosis of the jaw (BRONJ) has been reported. This is most commonly identified in the mandible. The pathogenesis of BRONJ is unknown, but vascular injury with ischemia or inhibition of osteoclasts by the drugs have been postulated. We report a series of patients with BRONJ and compare the histologic findings to those seen with osteoradionecrosis.
**Design:** 23 patients with documented bisphononate-related osteonecrosis were studied. The specimens were examined histologically for bony necrosis, acute and chronic inflammation including plasma cells, osteoclasts, osteoblasts, bony remodeling, fibrosis, and bacteria. Our comparison group consisted of 9 patients with mandibular osteoradionecrosis.
**Results:** 23 patients with mandibular osteonecrosis and prior use of bisphosphonate therapy were identified. The patients included 13 females and 10 males. Ages ranged from 48-80 with a mean of 63 years old. 21 patients were given zoledronic acid and 2 patients were given pamidronate and zoledronic acid. Adequate tissues for histologic examination were available for 21 patients with a total of 23 specimens examined. All specimens showed bone necrosis along acute and chronic inflammation and bacteria (predominantly actinomyces). An interesting finding was the presence of plasma cells which were more prominent in bisphosphonate-exposed patients. The presence of osteoclasts, osteoblasts, and bony remodeling were more commonly seen in bisphosphonate-exposed patients.
**Conclusions:** The pathology of mandibular bisphosphonate osteonecrosis is similar to osteoradionecrosis with the exception that the bisphosphonate-exposed specimens showed evidence of increased osteoclasts and osteoblasts as well as evidence of bony remodeling and plasma cell infiltrates. While the pathogenesis of bisphosphonate-related osteonecrosis remains unknown, our data favor vascular compromise as a mechanism.

| | Bisphosphonate Necrosis | Radiation Necrosis |
|---|---|---|
| Boney Necrosis | 100% | 100% |
| Osteoclasts | 70% | 22% |
| Osteoblasts | 65% | 22% |
| Boney remodeling | 78% | 44% |
| Acute/Chronic Inflammation | 100% | 100% |
| Plasma Cells | 87% | 33% |
| Bacterial Organisms | 100% | 100% |

### 1096 Sebaceous Epithelial-Myoepithelial Carcinoma of the Salivary Gland: A Report of 6 Cases of a New Histologic Variant

*T Nagao, A Shinozaki, M Fukayama, K Mukai.* Tokyo Medical University, Tokyo, Japan; University of Tokyo, Tokyo, Japan.
**Background:** Epithelial-myoepithelial carcinoma (EMC) of the salivary glands is an uncommon, low-grade malignant tumor. A recent report demonstrates sebaceous differentiation in this tumor even though its significance has never been documented as a precise histologic variant.
**Design:** Six cases of EMC exhibiting sebaceous differentiation (sebaceous EMC) of the parotid gland were analyzed for their clinicopathologic features and immunohistochemical characteristics. In addition, primary salivary sebaceous carcinomas were also examined for comparison.
**Results:** In our series, the incidence of sebaceous EMC was 0.2% among 3,012 cases of parotid gland tumors and 14.3% of all EMC cases. The 6 patients comprised 2 men and 4 women, ranging in age from 77 to 93 years (mean, 83.7 years). Neither cervical lymph node nor distant organ metastases were found in any cases of sebaceous EMC and no patients died of disease, though local recurrences developed in 1 patient. Conversely, cervical lymph node metastasis was detected in 2 of 3 patients with sebaceous carcinoma, one of whom died of disease at 12 months. Histologically, all 6 tumors had an area of sebaceous differentiation admixed with features of bi-layered ductal structures typical of EMC. A component of sebaceous differentiation was distributed diffusely in 4 tumors and focally in 2. Cytologic atypia of sebaceous EMCs was lesser than that of sebaceous carcinomas. Immunohistochemically, putative myoepithelial markers such as alpha-smooth muscle actin, calponin, p63, cytokeratin 14, S-100 protein, and vimentin were highly expressed in sebaceous EMC. However, the expression of the latter 4 markers was also observed in primary sebaceous carcinomas, whereas these tumors were all negative for alpha-smooth muscle actin and calponin. Positive immunoreactivity for epithelial membrane antigen, adipophilin, and perilipin confirmed sebaceous differentiation in EMC.
**Conclusions:** Sebaceous EMC is a low-grade malignancy, similar to conventional EMC. Our data also suggest that immunohistochemical examination of specific myoepithelial markers is helpful in distinguishing sebaceous EMC from sebaceous carcinoma, an aggressive tumor.

### 1097 Minor Salivary Gland Tumors of the Larynx, a Clinicopathologic Study of 33 Cases

*CJ Nero, CL Wu, CN Powers, JF Krane, WC Faquin.* Massachusetts General Hospital, Boston, MA; Virginia Commonwealth University Medical Center, Richmond, VA; Brigham and Women's Hospital, Boston, MA.
**Background:** Minor salivary gland tumors of the larynx are rare, comprising less than 1% of laryngeal neoplasms. Using a series of 33 cases, we report our experience with this rare set of tumors - their histomorphologic features, clinical behavior, and clinical follow-up.
**Design:** 33 laryngeal salivary gland tumors were retrieved from the archival pathology records at three academic institutions between the years 1976 and 2007. There are 9 females and 24 males with an average age of 57 years (range: 18 to 81 years) in this study. Two pathologists reviewed the available slides, and correlated histopathology, clinical findings, management and follow-up.
**Results:** Of 33 laryngeal minor salivary gland tumors, 32 (97%) were malignant (adenoid cystic carcinoma N=18, mucoepidermoid carcinoma N=12, adenocarcinoma NOS N=2) and 1 (3%) was benign (pleomorphic adenoma, N=1). 88% of mucoepidermoid carcinomas were intermediate to high grade. Fifteen tumors (45%) were located in the glottis, 15 (45%) in the subglottis, and 3 (9%) in the supraglottis. Where clinical information was available, 22 (85%) had surgery (5 with adjuvant radiotherapy), and 4 (15%) had radiotherapy and/or chemotherapy. Of the patients who were treated surgically, 77% underwent laryngectomy while 23% received partial resections; 60%

of total cases had positive margins. In clinical follow-up, 69% of the patients with adenoid cystic carcinoma and 37% with mucoepidermoid carcinoma developed local or distant recurrences.

**Conclusions:** Minor salivary gland tumors involving the larynx are rare, and when they occur, most are malignant. Despite aggressive clinical management that in a majority of cases included laryngectomy, there is a high propensity for local and distant recurrence.

### 1098 Eosinophilic Angiocentric Fibrosis: Clinicopathologic Characteristics and Potential Pitfalls

*ED O'Sullivan, R Jain, WC Faquin, CN Powers.* Virginia Commonwealth University, Richmond, VA; Massachusetts General Hospital, Boston, MA.

**Background:** Eosinophilic angiocentric fibrosis (EAF) is an extremely rare disease of unknown etiology that occurs mainly in the sinonasal tract. Though the treatment of choice for EAF is not clear, surgery is an acceptable treatment modality. Thus, it is important to distinguish EAF from other lesions that may have similar overlapping clinical and histological presentations: chronic sinusitis, allergic sinusitis, Wegener granulomatosis, granuloma faciale, Kimura disease, and malignant lymphomas.

**Design:** We report three cases of EAF from two academic institutions to highlight the important clinicopathologic features and potential pitfalls of this rare entity.

**Results:** Aside from the generic presentation of sinonasal obstruction, there was no distinctive clinical symptom suggestive of EAF. Patient A, a 57 year old male, with a history of allergies and wood dust exposure, presented with a decreased sense of smell and mild peripheral eosinophilia. Patient B, a 27 year old female, had a history of prior cocaine abuse. Patient C, a 31 year old female, presented with proptosis. The radiologic findings in patients A and B showed expanding soft tissue masses in the nasal turbinates that involved the sinuses, and in patient C, a right orbital mass extending into the sinuses. Indirect immunoflourescence testing for anti-neutrophilic cytoplasmic antibodies (ANCA) was negative in all patients. Lymphoma work-up performed in patients A and B was negative. Histologically, all cases demonstrated a dense fibrotic stroma with a perivascular whorling pattern, and a lymphoplasmacytic and eosinophilic infiltrate. In addition, patient C demonstrated modest acute inflammation and a focal endothelial proliferation. No vasculitis, granuloma formation, giant-cell histiocytic reaction, or necrosis were identified in any of the cases.

**Conclusions:** Although the histologic features of EAF have been documented in sporadic case reports and small series, this disease still remains problematic. The dense fibrotic stroma with perivascular collagen whorling, the characteristic feature of EAF, is not seen in the more prevalent allergic/chronic sinusitis. The absence of giant cells, granulomas, and necrosis may help to distinguish EAF from granuloma faciale. Ancillary studies may be useful to exclude other histologically similar diseases such as Wegener granulomatosis.

### 1099 Brachyury, SOX-9, and D2-40, the Next Generation Markers in the Skull Base Chordoma vs Chondrosarcoma Differential: End of Discussion?

*GJ Oakley, K Fuhrer, RR Seethala.* University of Pittsburgh, Pittsburgh, PA.

**Background:** The distinction between chondrosarcoma (CHS) and chordoma (CD) of the skull base/head and neck is prognostically important, however both have sufficient morphological overlap to make distinction difficult. CD can be stratified into conventional chordoma (coCD) and chondroid chordoma (chCD), the latter composed of classic chordoma admixed with areas of cartilaginous differentiation adding further to the diagnostic challenge. Traditionally, cytokeratins positivity has been used to discriminate CD from CHS. As a result of expression profiling studies, additional candidate markers have emerged to help in this distinction. We sought to evaluate the performance of "next generation" markers: Brachyury, SOX-9, and D2-40 in differential diagnosis of skull base/head and neck chondroid tumors.

**Design:** Paraffin blocks from 104 skull base/head and neck chondroid tumors from 70 patients were retrieved. Gold standard diagnoses were made based on morphology and/or whole section immunohistochemistry for a cytokeratin and S100 yielding: 80 CD (45 chCD and 35 coCD) and 24 CHS. A tissue microarray block containing 0.6 mm cores of each tumor in triplicate along was constructed using a manual array (MTA-1, Beecher Instruments, Sun Prairie WI). Antibodies used for immunohistochemical staining were: Cytokeratin AE1/AE3, S100, Brachyury, SOX-9, and D2-40. For visualization of staining, the ImmPRESS detection system (Vector Laboratories, Burlingame, CA) with DAB substrate was used. Sensitivities and specificities were calculated for each marker.

**Results:** Core loss from the microarray ranged from 25-28.9% yielding 74-78 viable cases per stain. The performance characteristics of antibodies are summarized in table 1.

Performance Characteristics

| Marker | Sensitivity % | Spec % | N |
|---|---|---|---|
| AE1/AE3 | 96.7 | 100 | 78 |
| Brachyury | 89.7 | 100 | 74 |
| SOX-9 | 94.1* | 1.7* | 77 |
| D2-40 | 77.8* | 85.7* | 74 |
| AE1/AE3+Brachyury | 98.3 | 100 | 78 |

*with respect to CHS

All cases were S100 positive. Both CD that were negative for AE1/AE3 were chCD. Only 3/6 (50%) Brachyury negative CD were chCD. Of the D2-40 positive CD, 7/8 (82.5%) were chCD.

**Conclusions:** Cytokeratin is still the most sensitive marker of CD, though when combined with brachyury, sensitivity improves slightly. SOX-9 is apparently common to both notochordal and cartilaginous differentiation and is not useful in the CD-CHS differential diagnosis. D2-40 still remains the only positive marker for CHS, though its accuracy is less than previously reported.

### 1100 Frequent Promoter Hypermethylation of Retinoic Acid Receptor Responder Gene-1 (RARRES-1) in Oral Leukoplakia: A Potential Epigenetic Marker To Predict Malignant Transformation

*DC Phan, C Xie, L Li, RF Schaefer, M Kokoska, BR Smoller, CY Fan.* University of Arkansas for Medical Sciences, Little Rock, AR; John L. McClellan Memorial Veterans Hospital, Little Rock, AR.

**Background:** Oral leukoplakia is most commonly caused by cigarette smoking and alcohol use. Oral leukoplakia is wildly considered as a preneoplastic lesion, with up to 15% of them eventually progressing to invasive squamous cell carcinoma (SCC). Epigenetic alterations in association with promoter hypermethylation are one important mechanism of silencing tumor-suppressor gene in human cancer. RARRES-1 gene is a tumor-suppressor gene and has been shown that RARRES-1 gene is silenced by promoter hypermethylation in a wide variety of human cancers, including head and neck cancer. In this study, we analyzed 70 cases of oral leukoplakia to determine the frequency of aberrant promoter methylation and its potential use as epigenetic marker for prediction of malignant transformation.

**Design:** A total of 120 cases of oral leukoplakia with or without dysplasia and in situ or invasive SCC from 68 patients were included. In 31 patients, multiple consecutive biopsies with natural progression from leukoplakia to invasive SCC obtained at various time points were obtained. DNA samples from these lesions were extracted and subjected to methylation-specific PCR using a primer set specific for RARRES-1 gene promoter.

**Results:** RARRES-1 promoter hypermethylation was detected in 24 of 68 (35%) patients with oral leukoplakia. Among 31 patients with multiple consecutive biopsies, 15 patients demonstrated positive RARRES-1 promoter methylation in their initial biopsies of oral leukoplakia without dysplasia. Three patients subsequently developed dysplasia in relapsed leukoplakia while 12 showed progression to invasive SCC. Among 16 patients who did not have RARRES-1 promoter methylation in their initial oral leukoplakia, 8 showed progression to invasive SCC and 8 displayed no evidence of malignant progression despite multiple relapses of oral leukoplakia.

**Conclusions:** RARRES-1 promoter hypermethylation occurs frequently in oral leukoplakia, indicative of the important role of RARRES-1 gene in oral squamous carcinogenesis. The presence of RARRES-1 promoter methylation in initial oral leukoplakia may have predictive value for subsequent development of dysplasia or invasive SCC.

### 1101 Idiopathic Orbital Inflammation (IOI) with IgG4 Positive Plasma Cells: Orbital Manifestation of IgG4-Related Disease

*JA Plaza, J Garrity, T Witzig, A Ananthamurthy, A Dogan, D Salomao.* Mayo Clinic, Rochester, MN.

**Background:** IgG4-related disease, recently recognized entity, is characterized by abundant IgG4 positive plasma cell tissue infiltration and high serum IgG4 levels. It is considered an autoimmune disease, responsive to steroid therapy. It can manifest as tumor-like lesions involving several organs. Inflammatory pseudo-tumors also occur in the orbit and lacrimal glands and are called idiopathic orbital inflammation (IOI) because of their unknown etiology. They are also treated with steroids. Our aim was to determine presence of IgG4 plasma cells, Epstein Barr virus (EBV) and ALK-1 in IOI and their potential role in the pathogenesis of this process.

**Design:** 25 patients (pts) with IOI identified from Mayo Clinic Rochester files, January 2003 to December 2006. Clinical/radiological findings were reviewed. Tissues (ts) evaluated for presence/amount of fibrosis, follicular hyperplasia and cell type infiltrate. Immunohistochemistry for IgG4, ALK -1 and in situ hybridization for EBV (EBV-ISH) were performed. Immunophenotyping by immunohistochemistry/flow cytometry were done in 22 cases (cs) and molecular genetics for immunoglobulin and T-cell receptor gene rearrangements in 5.

**Results:** 17 women and 8 men (mean age 55 yr, range 30-71). Presenting symptoms included mass and/or proptosis (84%), visual symptoms (60%) and pain (12%). MRI/CT showed enlarged lacrimal gland (77%), involvement of extra-ocular muscle (50%), orbital soft tissue (20%), intracranial extension (12%) and bilateral disease (32%). Ts showed moderate to marked lymphocytic infiltrate in 23 cs (92%) and follicular hyperplasia in 21 (84%; marked 52%). Fibrosis was present in 15 cs (60%, marked 13%). Plasma cells were seen in all cs and eosinophils in 7. IgG4 positive plasma cells were present in 13 cs, all with background fibrosis. Of these 13 pts, one was tested and had elevated serum IgG4 levels. EBV-ISH and ALK-1 were negative in all cs. Lymphoma was excluded by immunophenotyping or molecular genetics.

**Conclusions:** 52% of our cases of IOI were associated with IgG4 positive plasma cells microscopically. One pt tested had elevated IgG4 serum levels. EBV and ALK-1 were not identified. These findings suggest an autoimmune etiology for this mass-producing inflammatory process in the orbit/lacrimal gland. Although non-specific, presence of IgG4 positive plasma cells in tissue biopsy is helpful feature in making histological diagnosis. The role of an elevated serum IgG4 as marker of this disease clinically needs further investigation.

### 1102 Human Papillomavirus and p16 Status in 438 Head and Neck Squamous Cell Carcinomas

*MC Policarpio-Nicolas, MH Stoler, EB Stelow, CA Moskaluk, SM Hong, HF Frierson, Jr.* University of Virginia Health System, Charlottesville, VA.

**Background:** HPV has been implicated as a risk factor for head and neck cancer in addition to the widely accepted risk factors of alcohol and tobacco use. Particularly, head and neck squamous carcinomas (HNSCCs) that arise in the oropharynx have a strong association with HPV. The data regarding this, however, have varied, likely due to the various sensitivities and specificities of the HPV testing modalities used. The aim of this study is to determine HPV and p16 status in a large series of HNSCCs, to compare the statuses of these two markers and to identify any difference in expression between these markers in oropharyngeal and non-oropharyngeal HNSCCs.

**Design:** Tissue microarrays were constructed from 438 archival formalin-fixed, paraffin-embedded HNSCCs from the following anatomic locations: oral cavity (174), mandible (2), salivary gland (12), nasal cavity (17), pharynx (25), larynx (187) and lymph node metastases (21). Automated in-situ hybridization for HPV was performed on the tissue microarrays using the Ventana HR HPV III probe set. Immunohistochemistry was performed using an antibody to $p16^{INK4}$ (clone G175-405, BD Pharmingen). Strong nuclear and cytoplasmic staining was scored as positive for p16 as follows: negative if 0 to <5% cells stained and positive if >5% of the cells stained.
**Results:** Of the 438 cases, 12 had HPV by ISH and 50 were p16 immunoreactive. Seven of twelve HPV-positive tumors were from oropharynx (3 tongue, 2 tonsil, 2 oropharynx) and three were from the larynx. Of the fifty cases which were p16 immunoreactive, twenty-four (48%) were oropharyngeal (8 tongue, 7 tonsil, 2 floor of mouth, 2 gingival, 2 maxilla, 2 oral cavity site unspecified and 1 lip), twenty-two (44%) were laryngeal, 2 (4%) mandibular, 1(2%) nasal and 1 (2%) was from the parotid. Only two cases were HPV positive and p16 nonreactive (tonsil and larynx).
**Conclusions:** P16 expression is strongly correlated with the presence of HPV by ISH. Forty HPV-negative tumors nonetheless exhibited p16 overexpression suggesting either false positive p16 immunorectivity with regards to HPV or a low sensitivity for HPV ISH (perhaps secondary to low tumor viral titer or infection by other non-crossreactive HPV subtypes). Given that the site distributions are similar, the latter seems more likely and we conclude that p16 immunoreactivity may be a good surrogate marker for the detection of HPV. Using this marker, 11% of our HNSCCs were related to HPV infection and the majority were from either the oropharynx or larynx.

### 1103 Mitochondrial DNA 4977 Basepair Deletion Occurs More Frequently in Oral Leukoplakia Than in Head and Neck Squamous Cell Carcinoma: Its Potential Role as Tumor Suppressor in Head and Neck Squamous Carcinogenesis

*MC Quick, C Xie, L Li, RF Schaefer, M Kokoska, BR Smoller, CY Fan.* University of Arkansas for Medical Sciences, Little Rock, AR; John L. McClellan Memorial Veterans Hospital, Little Rock, AR.
**Background:** Mitochondria are the primary source of free radicals, which in turn causes mitochondrial DNA damages (mutation or deletion) and dysfunction. Mitochondrial DNA deletions occur frequently in the process of aging and malignant transformation. The most frequently occurred human mitochondrial DNA deletion is delta mtDNA 4977. Delta mtDNA 4977 has been characterized in a variety of human cancer and multiple studies show that this type of deletion occurs much less frequently in invasive cancer than in adjacent normal tissue, supporting a protective role of delta mtDNA 4977 in malignant transformation in human. In this study, we analyze delta mtDNA 4977 in 38 cases of oral leukoplakia and 136 cases of head and neck squamous cell carcinoma in an attempt to illustrate the frequency and possible role of delta mtDNA 4977 in head and neck squamous carcinogenesis.
**Design:** A total of 38 cases of oral leukoplakia and 136 cases of head and neck squamous cell carcinoma (HNSCC) were included. A complete clinical follow-up was available in 136 HNSCC patients. DNA samples from these lesions were extracted and subjected to PCR amplification using a primer set that franks the breakpoint of delta mtDNA 4977.
**Results:** Delta mtDNA 4977 was detected in 8 of 38 (24%) cases of oral leukoplakia and 6 of 136 (4.4%) cases of HNSCC. Clinical characteristics of HNSCC with or without delta mtDNA 4977 were then compared. Among 6 HNSCC containing delta mtDNA 4977, 5 (83%) are of T1-2, 6 (100%) are of N0 and 5 (83%) are of clinical stage I-II. By contrast, among 130 HNSCC without delta mtDNA 4977, 71 (54%) are of T1-2, 81 (62%) are of N0 and 50 (38%) are of clinical stage I-II.
**Conclusions:** Delta mtDNA 4977 occurs much more frequently in preneoplastic oral leukoplakia (24%) than in invasive HNSCC (4.4%). A possible tumor-suppressor function of delta mtDNA 4977 appears to be supported by our results that the frequency of delta mtDNA 4977 decreases dramatically in invasive HNSCC and that the HNSCCs that retain the delta mtDNA 4977 are correlated with smaller primary tumor (T1-2), negative neck disease (N0) and lower overall clinical stage (Stage I=II).

### 1104 Deletion of 1p32-p36 Is the Most Frequent Genetic Change and a Poor Prognostic Marker in Adenoid Cystic Carcinoma of the Salivary Glands

*PH Rao, D Bell, D Roberts, YJ Zhao, RS Weber, AK El-Naggar.* Texas Children's Hospital, Baylor College of Medicine, Houston, TX; M.D. Anderson Cancer Center, Houston, TX.
**Background:** Adenoid cystic carcinoma (ACC) is one of the most common and relentless malignancy of the submandibular and minor salivary glands. Attempts to identify molecular and biomarkers for this entity have yet to yield a clinically relevant factor. Therefore, a search for biological markers other than tumor stage traditional clinicopathologic factors is a subject of interest.
**Design:** We used a genome-wide screening method – comparative genomic hybridization (CGH) to identify DNA copy number changes in 53 primary tumors from equal number of patients with ACC. We correlated these DNA copy number changes with clinico-pathologic factors using Pearson's Chi-square or by the two-tailed Fisher exact test. The disease-specific survival and disease-free intervals were generated by the Kaplan-Meier product limit method. We applied array CGH and fluorescence in situ hybridization to validate the genetic findings.
**Results:** Chromosomal losses (n=134) were more frequent than gains (n=74). Underrepresentation of chromosomal regions was noted at 1p, 6q, 12q, 19, 9p, 13q and 17p. Strikingly, loss of 1p was found in 44% of the cases and it was restricted to the 1p32-p36 region. The other common regions of deletions were mapped to 6q23-q27, and 12q12-q14 regions. The most frequently gained chromosomal regions were on chromosomes 8 and 18. Of the genomic alterations with prognostic significance, only loss of the 1p32-p36 region was correlated with poor outcome.
**Conclusions:** Our study demonstrated that loss of 1p32-p36 is a frequent alterations with adverse biological significance in ACC. This specific genetic alteration will aid in the identification of putative tumor suppressor gene(s) at 1p32-p36. Molecular characterization of this chromosomal region utilizing the currently available genomic technologies will provide new insights into the biology and clinical behavior of ACC.

### 1105 Reproducibility of Grading in Salivary Gland Mucoepidermoid Carcinoma and Correlation with Outcome: Does System Really Matter?

*RR Seethala, AP Hoschar, A Bennett, AV Arrossi, JM Davison, AM Krasinskas, JL Hunt.* University of Pittsburgh, Pittsburgh, PA; Cleveland Clinic, Cleveland, OH.
**Background:** The grading of salivary gland mucoepidermoid carcinoma (MEC) is important prognostically and therapeutically. The use of a point based grading system has been previously shown to be more reproducible than one's "own" grading system. For the first time, we compare the reproducibility and correlation with outcome of three major grading systems as well as one's "own" grade in a set of 20 MEC.
**Design:** Twenty MEC spanning all grades were retrieved. Mean patient age was 53.1 (range: 15 -81) with a female predilection of 2.3:1. Site groups were: oral salivary -8, parotid -7, sinonasal mucoserous -3, and submandibular -2. Known stage groupings (n=15) were: Stage I/II – 10, Stage III/IV – 5. Five patients died of disease; mean follow up on survivors was 6.6 years. Slides were circulated to three head and neck pathologists and four non-head and neck pathologists for review. Individual histologic parameters were recorded and four grades were assigned: ones "own grade", Brandwein grade, AFIP grade, and Healy grade. Statistical analysis was performed using SPSS software (SPSS, version 14.0.0).
**Results:** Results are summarized in table 1.

Interobserver agreement and correlation with outcome for grading systems

| | "Own" grade | Brandwein | AFIP | Healy |
|---|---|---|---|---|
| Mean kappa for all pairs(range) | 0.37 [fair] (0.12 – 0.62) | 0.52 [moderate] (0.23 – 0.76) | 0.61 [substantial] ( 0.49 – 1.00) | 0.70 [substantial] (0.46 – 0.92) |
| Mean log rank p-value for all observers(range) | 0.035 (0.003 – 0.173) | 0.074 (0.002 – 0.229) | 0.001 ( >0.001 – 0.003) | 0.006 (0.001 – 0.033) |
| Number of "dead of disease" events in low grade MEC category | 5 | 0 | 10 | 3 |

**Conclusions:** Standardized schemas improve the reproducibility of grading regardless of subspecialty training. Interestingly, in our small set, AFIP grade and Healy grade were more reproducible and had better correlation with disease survival than the Brandwein grade. However, Brandwein grade more successfully predicted an indolent course for low grade MEC. Thus AFIP and possibly the Healy systems appear to "downgrade" tumors. An ideal grading system that most accurately predicts outcome and maintains low grade MEC as an indolent category is still lacking.

### 1106 The Value of p53 Expression in Salivary Mucoepidermoid Carcinoma by Immunohistochemistry and Fluoresence In Situ Hybridization

*RR Seethala, X Zhang, W Lang, JL Hunt.* University of Pittsburgh, Pittsburgh, PA; Cleveland Clinic, Cleveland, OH.
**Background:** The prevalence and prognostic value of p53 protein expression and *TP53* gene alterations (losses or mutations) in salivary gland mucoepidermoid carcinoma (MEC) vary greatly from study to study. To clarify the importance of p53 expression in MEC, we evaluate 20 tumors by p53 immunohistochemistry (IHC) and fluorescence in-situ hybridization (FISH) and extend the IHC analysis to 81 well characterized MEC.
**Design:** Twenty MEC were selected for whole section IHC analysis and FISH. Anti-p53 (1:100, clone DO-7, Dako, Carpinteria, CA) staining was performed and visualized using a brown (DAB) chromogen and was graded as strong, weak or negative. Dual color FISH analysis was performed using SpectrumGreen-labelled CEP17 and SpectrumOrange-labelled 17p13.1 (*TP53*) probes (Vysis, Downers, IL) on all cases that were immunohistochemically p53 positive. Cases were counted as positive for *TP53* deletion if >10% of diploid cells showed loss. Immunohistochemical staining was also performed on a tissue microarray (TMA) containing 81 MEC arrayed in triplicate (0.6 mm cores) using a manual arrayer (Beecher Instruments, Sun Prairie, WI). Staining was correlated with clinicopathologic parameters. Calculations were performed using SPSS software (SPSS version 14.0.0).
**Results:** In the initial set of 20 tumors, p53 immunopositivity was noted 22.2% of low/intermediate grade MEC, 45.5% of high grade MEC. Strong positivity was only present in 18.2% of high grade MEC. FISH showed *TP53* deletion in 2/5 (40%) high grade MEC. One FISH positive case showed strong staining, while the other showed weak staining. On the TMA, strong p53 positivity was far more frequently seen in high grade MEC (22.2%) than in low/intermediate grade MEC (2.2%) (p=0.009), and in stage III/IV MEC (21.7%) as compared to stage I/II MEC (2.4%) (p=0.020). Significant univariate predictors of disease specific survival included: Age>55 (log rank p<0.001), stage (p<0.001), grade (p<0.001), margin status (p=0.006), and strong P53 positivity (p=0.044). On multivariate analysis P53 was not an independent prognosticator.
**Conclusions:** Strong P53 immunoreactivity is largely restricted to high grade or high stage MEC. However outside these correlations, there is no independent prognostic value of p53 IHC staining in MEC. By initial FISH analysis, a subset of p53 positive tumors show *TP53* deletions as the mechanism of p53 alteration in high grade MEC which warrants further investigation.

### 1107 Undifferentiated Carcinomas of the Upper Aerodigestive Tract Associated with NUT Rearrangements

*EB Stelow, AM Bellizzi, SE Mills, JL Kutok, JC Aster, RD LeGallo, CA French.* University of Virgina, Charlottesville, VA; Brigham and Women's Hospital, Boston, MA.

**Background:** Undifferentiated carcinomas (UCs) of the upper aerodigestive tract occur most frequently within the nasopharynx and are associated with infection by Epstein-Barr Virus (EBV). An unusual group of aggressive carcinomas are characterized by translocations that involve *NUT*, a novel gene on chromosome 15. In about two thirds of cases, *NUT* is fused to *BRD4* on chromosome 19. These tumors are undifferentiated, have focal squamous differentiation, and are reported to occur in children and young adults. This study investigated the prevalence of *NUT* rearrangement and the diagnostic significance of NUT expression in a series of upper aerodigestive tract UCs. The histologic features of these tumors are described.

**Design:** All UCs of the upper aerodigestive tract not associated with EBV seen over a sixteen year period were reviewed. Clinical and histologic features were noted. Additional material was submitted for fluorescent in situ hybridization (FISH) using split-apart probes to the *NUT* and *BRD4*. Immunohistochemistry (IHC) was performed on all cases using a polyclonal antibody to NUT, and on select cases with antibody to p63.

**Results:** Thirty-one UCs (13 females, 18 males; age range 15-88) of the upper aerodigestive tract were identified that had material available. Twenty-five tumors had originally been diagnosed as sinonasal undifferentiated carcinomas. Five of 28 cases (2 males, 3 females; ages 31, 39, 40, 47 and 78 years) with interpretable results showed rearrangements of the *NUT* and *BRD4* by FISH. Four of these 5 cases showed diffuse (>90%) nuclear expression of NUT by IHC; all other tumors showed at most focal (<50%) nuclear expression of NUT. UCs with *NUT* gene rearrangement had focal abrupt squamous differentiation in 2 cases and intense and diffuse immunoreactivity with antibody to p63 in 4 cases.

**Conclusions:** Approximately 20% of UCs of the upper aerodigestive tract not associated with EBV were found to have rearrangements of *NUT* by FISH. IHC using NUT antibody may be a useful method for the identification of these tumors. Abrupt squamous differentiation and / or p63 immunoreactivity is seen with these malignancies. Though previous reports suggest that *BRD4-NUT* carcinomas afflict only children and young adults, four of five of the patients described are mature adults older than any heretofore reported, suggesting that previous reports may have been biased in their case selections.

### 1108 Real Time Quantitative RT-PCR Analysis of High Mobility Group A2 (HMGA2) mRNA Expression Assists in Distinguishing Benign from Malignant Thyroid Tumors

*K Swartz, L Jin, LA Erickson, TJ Sebo, J Zhang, RV Lloyd.* Mayo Clinic, Rochester, MN.

**Background:** The distinction between benign and low grade malignant thyroid tumors in histological and cytological preparations can be very difficult. HMGA2 is a member of the high mobility group protein family which is involved in the regulation of chromatin function. HMGA2 is overexpressed in some carcinomas including thyroid tumors.

**Design:** We used real time quantitative RT-PCR for HMGA2 mRNA to analyze gene expression in 109 cases of thyroid tumors. These included formalin-fixed paraffin embedded tissue samples of follicular adenomas (FA) (n=31) and follicular variant of papillary thyroid carcinomas (FVPTC) (n=37) and cytological preparations of FA (n=8), follicular carcinomas (FCA) (n=9) and papillary thyroid carcinomas (PTC) including both classical (n=17) and FVPTC (n=7). All diagnoses were confirmed in histological sections after surgery. Statistical analysis was done using logistic regression with receiver operating characteristic analysis.

**Results:** Comparison of FA and FVPTC from paraffin sections used a cut-off point of 7.1-fold increase to separate benign and malignant tumors with a specificity of 100% and a sensitivity of 91.9%. All FA were classified as benign, while 34/37 (91.9%) of the FVPTC were classified as malignant. Analysis of cytologic specimens used a cut-off point of 11.6-fold increase to separate benign and malignant tumors with a specificity of 100% and a sensitivity of 93.9%. All FA were classified as benign, while 31/33 (93.9%) of the other tumors (FCA, FVPTC and PTC) were classified as malignant.

**Conclusions:** Real time quantitative RT-PCR for HMGA2 mRNA expression can be used to assist in the distinction of benign and low grade malignant thyroid tumors in histologic and cytologic preparations.

### 1109 Differential Expression of *KRT14, MMP1, GAS1, COL6A2* Gene Proteins in Squamous Cell Carcinomas of the Head and Neck (HNSCC), and Lung (LSCC) Can Help Distinguish One from the Other

*G Tang, R Jaffar, K Dresser, M Zona, HT Jaffar, ML Prasad.* University of Massachusetts Medical Center, Worcester, MA; Citizen Bank Financial Group, Cranston, RI.

**Background:** Distinguishing metastatic HNSCC to the lung from primary LSCC is critical for tumor staging and management. Vachani et al (Clin Cancer Res 2007;13:2905) using oligonucleotide microarrays, identified 10 genes, which showed significantly different expressions in HNSCC and LSCC. In this study, we attempt to detect the expressions of these gene proteins using immunohistochemistry (IHC), and evaluate their utility in differentiating HNSCC from LSCC.

**Design:** We performed IHC on tissue microarrays of HNSCC (58 cases, UMass Memorial Medical center) and LSCC (61 cases, Cybrdi, Frederick, MD) using antibodies against 8 of the 10 gene proteins (CK14, MMP1, GAS1, COL6A2, KLK10, SFTPB, TPM3, MYH2) identified by Vachani et al in their publication. Positive expression was defined as cytoplasmic, membranous and/or nuclear staining in >10% tumor cells for all proteins except COL6A2 for which staining in ≥30% tumor cells was considered positive.

**Results:** Six of the proteins showed significantly ($p < 0.05$) different expression in HNSCC and LSCC. CK14, MMP1, GAS1, COL6A2 and KLK10 showed more frequent expression in HNSCC whereas SFTPB was more frequently expressed in LSCC, consistent with the gene expression profiling data. CK14, MMP1, GAS1 and COL6A2 showed high sensitivity (>75%) for HNSCC. KLK10 and SFTPB had <20% sensitivity for HNSCC and LSCC respectively. CK14 and MMP1 showed strong intensity staining reactions in positive tumor cells. GAS1 and COL6A2 showed weak and focal staining reaction when positive.

CK14, MMP1, GAS1 and COL6A2 Expression in HNSCC and LSCC

| CK14 (n) | Positive | Negative | Sensitivity | Specificity | Accuracy |
|---|---|---|---|---|---|
| HNSCC (55) | 49 | 6 | 89% | 72% | 81% |
| LSCC (61) | 17 | 44 | | | |
| **MMP1 (n)** | | | | | |
| HNSCC (54) | 54 | 0 | 100% | 64% | 81% |
| LSCC (61) | 22 | 39 | | | |
| **GAS1 (n)** | | | | | |
| HNSCC (56) | 44 | 12 | 78.5% | 97% | 88% |
| LSCC (61) | 2 | 59 | | | |
| **COL6A2 (n)** | | | | | |
| HNSCC (53) | 46 | 7 | 86.8% | 98% | 93% |
| LSCC (61) | 1 | 60 | | | |

**Conclusions:** Immunohistochemical detection of CK14, MMP1, GAS1 and COL6A2 can help distinguish HNSCC from primary LSCC with a high degree of accuracy. The strong intensity immunostaining reaction of CK14 and MMP1 makes them easily interpretable and therefore, suitable for routine practice.

### 1110 Endolymphatic Sac Tumors: A Clinicopathologic Analysis of 5 Cases

*SY Wang, L Lin, SM Li, L Zhu.* Eye, Ear, Nose and Throat Hospital of Fudan University, Shanghai, China.

**Background:** Endolymphatic Sac Tumors (ELSTs) are rare locally aggressive neoplasms of petrous temporal bone with benign papillary glandular histologic appearance and may occur sporadically or associate with von Hippel-Lindau (VHL) disease.

**Design:** To study the clinicopathologic features, histologic diagnosis and differential diagnosis of ELSTs. Light microscopic examination and immunohistochemical investigation were performed in 5 cases of ELSTs.

**Results:** Amongst the 5 patients studied, 2 were males and 3 were females and their age ranged from 14 to 41 years old (mean age=31years). The commonest clinical presentations were tinnitus, vertigo and sensorineural deafness similar to Meniere's disease. Other symptoms could include nausea, vomintting, dizziness and headache. All patients had no stigmata of von Hippel-Lindau disease. Computed tomography and magnetic resonance imaging scans with enhancement revealed homogeneous mass in the posterior cranial fossa, vicinity of the jugular foramen, endolymphatic sac and cerebellopontine angle. Angiography demonstrated hypervascular lesions. Grossly, fragments were generally reddish and brownish in color. Histologically, these tumors were composed of uniform cuboidal cells with clear to acidophilic cytoplasm forming papillary architectures that rest on a vascular stroma. Some cases had hypercellular areas and crowded glandular structures, which showed cystic dilatation of the glands containing secretion resembled follicle-like appearance in thyroid tumors. One case had bone invasion. Pleomorphism, mitotic activity and necroses were very rare. Immunohistochemical studies demonstrated that tumor cells were positive for cytokeratin, epithelial membrane antigen, neuron-specific enolase and S-100 protein, one of them was positive for vimentin and glial fibrillary acid protein. All tumor cells were negative for thyroglobulin, transthyretin, synaptophysin and chromogranin. Follow-up information was available in all patients (17-42 months) and no local recurrence or metastasis.

**Conclusions:** ELSTs are rare and low-grade adenocarcinomas involving the inner ear. Even though they have been considered as benign pattern, they are characterized by destructive and infiltrative local growth. Correct diagnosis relies on exact site of origin and detailed pathologic assessment and application of ancillary investigations. The differential diagnoses include choroid plexus papilloma, jugulotymapanic paraganglioma, papillary meningioma, ceruminous gland tumor, middle ear adenoma and metastatic lesions from thyroid or renal cell carcinoma.

### 1111 Low Grade Intraductal Carcinoma: A Relative of Cystadenocarcinoma, Precursor to Salivary Duct Carcinoma, or Both?

*S Wayne, EL Barnes, RR Seethala.* University of Pittsburgh, Pittsburgh, PA.

**Background:** The histogenesis of low grade intraductal carcinoma (LGIC)/low grade cribriform cystadenocarcinoma is controversial. While the bulk of the literature has focused on the relationship (or lack thereof) of this entity to the salivary duct carcinoma (SDC), a potential link to cystadenocarcinoma (CCA) has not been explored. To this end, we compared the histologic and immunophenotypic features of LGIC and CCA.

**Design:** Eight cases of LGIC and 24 of CCA were retrieved from the pathology archives. Six cases were reclassified as LGIC and five as other tumor types (excluded from analysis) yielding 14 cases of LGIC and 13 of CCA. Clinical, demographic, histologic and immunohistochemical parameters were reviewed.

**Results:** Results are summarized in Table 1.

Table 1

| | LGCC | CCA | P value |
|---|---|---|---|
| N | 14 | 13 | |
| Age (SD) | 62.7 (10.8) | 70.3 (9.1) | NS |
| Gender (% female) | 57.1 | 76.9 | NS |
| Location | Parotid 14 | Parotid 8 Oral cavity 3 Oropharynx 1 Intraoss 1 | |
| Mean size cm (SD) | 1.3 (0.7) | 1.6 (0.7) | NS |
| Prominent cribriform/solid (%) | 71.4 | 15.4 | 0.006 |
| Epithelial pigment (%) | 50.0 | 15.4 | NS |
| Columnar cells (%) | 64.3 | 84.6 | NS |
| Mean % oncocytic areas (SD) | 1.5% (3.0) | 33.8% (38.9) | 0.013 |
| S100 (epithelium) | 9/9 | 4/5 | NS |
| P63/calponin/SMA | 8/8 | 4/6 | NS |
| Androgen receptor | 1/7 (focal) | 0/3 | NS |
| Her-2/Neu | 1/4 (focal) | 0/1 | NS |

Demographic parameters were similar between groups, although some CCA arose outside the parotid. Mitotic rate, nuclear grade, and presence of necrosis, angiolymphatic invasion, and perineural invasion did not differ between the groups. Both LGIC and CCA showed S100 epithelial positivity. Additionally all LGIC and most CCA exhibited a p63, SMA, and/or calponin positive outer myoepithelial layer. AR and Her-2/Neu were noted in one discrete focus of atypia in one LGIC but otherwise were negative. The only significantly different features were a prominent solid/cribriform growth pattern in LGIC and a large oncocytic component in CCA.

**Conclusions:** There is considerable clinical, morphologic and immunophenotypic overlap between LGIC and at least some tumors historically designated as CCA. Moreover, the S100+/AR- phenotype along with the low grade phenotype and predominance of a delimiting outer myoepithelial layer distinguishes both lesions from SDC. Hence, regardless of the ultimate prevailing nomenclature, LGIC may actually be more closely related biologically to a subset of CCA than to SDC. However, we show some evidence that rare LGIC may be able to progress to SDC.

### 1112 Trk-A, Trk-B, p75NRT and GRP78 Expression in Olfactory Neuroblastoma

*I Weinreb, D Goldstein, J Irish, B Perez-Ordonez.* University Health Network, Toronto, ON, Canada.

**Background:** TrkA, TrkB and p75NRT are neurotrophin receptors associated with numerous solid tumors, most notably pediatric neuroblastoma (NB). GRP78 is an endoplasmic reticulum protein, known to be associated wth differentiation of neuroblastic cells. TrkA, p75NRT and GRP78 overexpression are favourable prognostic factors in NB while TrkB is associated with higher grade and a poorer prognosis. Olfactory neuroblastoma (ONB) is a clinically distinct tumor with similarities to pediatric NB, however prognosis does not correlate well with histologic grade. TrkA and p75NRT have been shown to be expressed in ONB, but have not been correlated with clinical outcome. TrkB and GRP78 have not been previously investigated in ONB.

**Design:** An archival search was performed for ON and a total of 20 cases with sufficient clinical data and tissue were reviewed for diagnosis and Hyams grading. All cases were stained with TrkA, TrkB, p75NRT and GRP78. They were scored as negative, focally positive or positive, with nuclear staining recorded for TrkA/TrkB and cytoplasmic or memranous staining for p75NRT and GRP78.

**Results:** There were 20 patients (13 male and 7 female) with an age range of 20-79 years (average 49.3). They presented as left (9), right (6) or bilateral (5) nasal masses. The symptom duration ranged from 1-24 months (m) (average 10.3) and included anosmia, visual defects, nasal obstruction, epistaxis or headache. Three patients presented with neck masses. The patients were treated with surgery (4), surgery with preoperative radiation (10) or postoperative radiation (5) or radiation and chemotherapy without surgery (1). Follow up ranged from 3 to 152 m (average 58.6 m). Thirteen patients have no evidence of disease at an average of 59.4 m. Five patients are alive with persistent local disease at an average of 72.6 m, 2 of which have distant metastases. Two patients have died of disease at 3 and 33 m. The Hyams grade for the patients was 1 (4), 2 (10), 3 (5), 4 (1). Strong TrkA expression was seen in 18 cases (90%), TrkB in 17 (85%), p75NRT showed focal membranous staining in 12 (60%) and GRP78 was weakly expressed in 18 (90%). There was no correlation between Hyams grade or outcome with expression of the above markers.

**Conclusions:** ONB shows strong nuclear expression of TrkA and TrkB, focal membranous p75NRT and weak expression of GRP78. TrkA and TrkB may have a role in the pathogenesis of ONB, but unlike pediatric NB do not correlate with prognosis or level of differentiation.

### 1113 Serous Hamartomas of the Sinonasal Tract. Report of 6 Cases

*I Weinreb, D Gnepp, N Laver, A Hoschar, J Hunt, R Chetty, B Perez-Ordonez.* University Health Network, Toronto, ON, Canada; Brown University School of Medicine, Providence, RI; New England Medical Center, Boston, MA; The Cleveland Clinic, Cleveland, OH.

**Background:** Sinonasal serous hamartomas (SH) are rare lesions not widely recognized by surgical pathologists. This lack of recognition and the scanty information regarding their clinicopathologic spectrum and phenotype may lead to an unwarranted diagnosis of low grade sinonasal adenocarcinoma (LGSNA).

**Design:** 6 SHs were studied. Immunohistochemical stains for CK7, CK14, CK19, CK20, HMWK, p63, SMA, MSA, calponin, and S100 were performed in 4 cases. The staining was evaluated in lesional serous glands and respiratory epithelium and was scored as diffusely positive (+), or negative (-).

**Results:** There were 4 males and 2 females. Patients' age ranged from 53-85 years. Symptoms included nasal obstruction and epistaxis. A history of chronic sinusitis and bilateral polyposis was obtained in 1 and 2 patients respectively. Follow-up in 5 patients, ranging from 1-60 months, revealed no recurrences. The masses involved the posterior nasal septum (5) and middle meatus/lateral nasal wall (1). The lesions were polypoid and ranged in size from 2.6-4.0 cm. All cases were covered by respiratory epithelium. The stroma was edematous to fibrous and contained variable proportions of cysts or invaginated respiratory epithelium admixed with small, bland serous glands, ducts and tubules with lobular or haphazard patterns. In many foci, some serous glands arose from the cysts and invaginated respiratory epithelium. 3 cases had thickened basement membranes, 1 surrounding serous glands, and 2 around invaginated respiratory epithelium, one focally and 1 diffusely. A variable chronic inflammatory infiltrate was found in all cases. Focal mucous differentiation and clear cell change were found in 1 case each. No mitotic activity, necrosis, cellular atypia, bone invasion, papillary or cribriform patterns were seen in any cases. The surface and invaginated respiratory epithelium and the serous glands were CK7+, CK19+ and CK20-. The surface and invaginated respiratory epithelium had a basal cell layer + for HMWK and p63 but CK14-. The serous glands were S100+ (3 cases) but lacked myoepithelial or basal cells.

**Conclusions:** Sinonasal SHs are benign lesions predominatly located in the posterior nasal septum lacking the pathologic features of LGSNA. Some SHs have overlapping features with respiratory epithelial adenomatoid hamartomas.

### 1114 Expression of Epidermal Growth Factor Family of Receptors in Salivary Duct Carcinomas: Comparative Methods and Targeted Therapy

*MD Williams, MS Kies, RS Weber, AK El-Naggar.* M.D. Anderson Cancer Center, Houston, TX.

**Background:** Salivary duct carcinoma, a high-grade adenocarcinoma resembling breast carcinoma is known to over-express EGF and HER-2 growth factor receptors. The mechanisms leading to over-expression of these receptors is poorly defined. Because of the value of these markers as a treatment target, we evaluated their status in salivary duct carcinomas by several techniques for correlations in receptor expression to determine the method(s) of choice for their assessment.

**Design:** Sixty-nine salivary duct carcinomas with archival formalin-fixed paraffin blocks available at the University of Texas, M.D. Anderson Cancer Center formed this study. Tissue microarrays were used to analyze tumors for immunohistochemical expression of HER-2 and EGFR. In addition, EGFR and HER-2 gene copy number and corresponding chromosome 7 and 17 centromeres by fluorescence in situ hybridization (FISH) and real time PCR using taqman probes were performed. Immunohistochemistry was evaluated for membranous expression: 3+ =strong complete in ≥ 30%; 2+= weak complete ≥30%; 1+ partial staining of tumor cells.

**Results:** Eighteen of 69 tumors showed membranous HER-2 staining (10, +3; 4, +2; and 4, +1). Of these eight had HER-2 gene amplification by FISH (6, +3; 0, +2; and 2, +1). Also one of the 51 tumors negative by immunohistochemistry showed gene amplification ($p<0.001$). Real time PCR detected one of three HER-2 amplified tumors and one false positive (ratio of 2). Fifty-three of 66 tumors showed membranous EGFR staining (28, +3; 13, +2; 12, +1). Gene amplification for EGFR was not identified. Chromosome 7 hyperplyoidy (average ≥3 copies per cell) was present in 10 of 57(18%) cases without correlation to immunohistochemical expression ($p=0.8$).

**Conclusions:** Our study shows in salivary duct carcinoma: 1) HER-2 over-expression by immunohistochemistry is frequently associated with HER-2 gene amplification as identified by FISH, 2) Immunohistochemistry can be used to screen for negative tumors unlikely to have HER-2 amplification, 3) Combining immunohistochemistry with FISH allows for the identification of the subset of patients with HER-2 amplified tumors who may benefit from Trastuzumab (Herceptin) therapy, 4) The mechanism(s) for EGFR high expression by immunohistochemistry remain undetermined and are not secondary to gene amplification or hyperploidy of chromosome 7.

### 1115 Ber-EP4, CK1, CK7 and CK14 Are Useful Markers for Identifying Basaloid Squamous Carcinoma. A Study of 45 Cases

*RD Winters, S Naud, MF Evans, WE Trotman, P Kasznica, A Elhosseiny.* University of Vermont College of Medicine, Burlington, VT; University of Vermont, Burlington, VT.

**Background:** Diagnosis of basaloid squamous carcinoma (BSCC) currently relies on histologic criteria, with variable immunohistochemical results reported in the small series. We investigated whether a diagnostically useful immunohistochemical profile could be elucidated utilizing our large sample size, representing the largest cohort examined to our knowledge.

**Design:** In this study 45 cases of BSCC were compared to 34 site-matched cases of conventional squamous cell carcinoma (SCC). All original slides and reports were reviewed prior to any immunostaining and diagnoses confirmed. Each tissue block was stained with antibodies directed against cytokeratins CK1, CK7, CK14, proliferation markers p63, MIB-1, c-kit, human papillomavirus (HPV)-associated marker p16, and Ber-EP4, previously associated with BSCC. Cases were initially identified as positive or negative. Positive cases were further subdivided based on percentage of positive tumor cells, intensity of staining and any pattern of staining (e.g. peripheral vs. central).

**Results:** Ber-EP4 was the most specific single marker for identifying BSCC, identifying 82% (37/45) BSCC and 68% SCC (23/34). Similar results were achieved using the combination of negative CK14 and CK7, plus positive CK1; which identified 73% (33/45) BSCC and 88% (30/34) SCC. Also, proliferation markers p63, c-kit and MIB-1 were expressed in significantly higher percentage of BSCC compared to site-matched SCC. Positive staining with p16, used as a surrogate marker for HPV, was present in 71% (32/45) BSCC cases compared to 59% (20/34) SCC.

**Conclusions:** Ber-EP4 is a useful diagnostic marker for BSCC, positive in 82% of cases and 68% SCC. An alternative is the combination of cytokeratins CK14 and CK7, known to be negative, and CK1, known to be positive, which achieves accuracy of 73% BSCC and 88% SCC. The two diagnostic approaches were in agreement in 66% of cases, the two methods were equally accurate in the divergent cases. Increased expression of the

proliferation markers supports the concept that BSCC is a rapidly growing tumor. Results of p16 stains support an etiological link between BSCC and HPV; interestingly, HPV was present in significantly more BSCC than SCC in this study (P = 0.02).

### 1116 Fascin Over-Expression Is Associated with Dysplastic Changes in Sinonasal Inverted Papillomas – A Study of 32 Cases

*HH Wu, S Zafar, H Yee, L Chiriboga, Y Huan, BY Wang.* New York University School of Medicine, New York, NY; Mount Sinai Medical Center, New York, NY.

**Background:** Inverted papillomas (IP) can show malignant transformation in upto 14-18% of cases. Fascin is an actin binding protein which plays a role in cellular motility and adhesion. Over-expression of Fascin has been seen in association with malignant transformation and aggressive tumor behavior. We evaluated Fascin expression in 32 sinonasal IP by immunohistochemistry.

**Design:** 32 excisional biopsy specimens of the nasal cavity and sinuses were retrieved from 23 patients over a 4 year period (2004-2007) from 2 major medical centers. Patients (18 male and 5 female) ranged in age from 22 to 72 years (average 52.5 years). The tissue was formalin fixed and paraffin embedded. The extent of dysplasia was based on histology. Immunohistochemical staining was performed on 4µm thick tissue sections using a Fascin monoclonal antibody, clone 55k-2 (prediluted; Ventana, Tucson, AZ, USA). Appropriate positive and negative controls were performed along with the study cases.

**Results:** In normal epithelium, Fascin stains the basal layer. The immunohistochemical results of Fascin in IP were scored based on the percentage of positive cells and density as follows: 0 (<5-25%); 1 (25-50%); 2 (50-75%); 3 (>75%). Fascin positivity was seen in 15 of 32 (46.8%) specimens from 15 of 23 patients (65.2%). 6 of 23 patients had recurrences. Among 15 Fascin-positive samples, 9 cases scored 1 (corresponding to mild dysplasia); 3 cases scored 2 (mild to moderate dysplasia); and 3 cases scored 3 (moderate to severe dysplasia/carcinoma in situ). The groups that scored 2 and 3 each included one patient with recurrences. 3 cases of IP without histologic dysplasia were also positive for Fascin, however, chronic inflammation was present in these samples. 17 cases were negative for Fascin expression including 14 IP without histological dysplasia and 3 IP with mild dysplasia (Table).

**Conclusions:** Fascin over-expression is seen more often in dysplastic epithelium in sinonasal IP, which corresponds to the extent of morphologically neoplastic change. Increased Fascin in IP may be associated with tumor progression and malignant transformation.

### 1117 Frequent Promoter Hypermethylation of Retinoic Acid Receptor Responder-1 (RARRES-1) Gene in Head and Neck Squamous Cell Carcinoma (HNSCC)

*C Xie, L Li, M Kokoska, JY Suen, BR Smoller, CY Fan.* University of Arkansas for Medical Sciences, Little Rock, AR; John L. McClellan Memorial Veterans Hospital, Little Rock, AR.

**Background:** 5-year overall survival for HNSCC is approx. 50% and this survival rate has not changed for past 50 years, underscoring the importance of developing novel and more effective treatment modalities. Retinoid, a cellular differentiating agent, were previously used in several clinical trials for the treatment of head and neck leukoplakia and invasive SCC with disappointing results. It has been shown that some retinoid effector genes, such as retinoic acid receptor β and RARES-1 are silenced by promoter hypermethylation and this may partly contribute to the failed therapeutic effect of retinoid in the treatment of HNSCC. In this study, we analyze 136 HNSCC to determine the frequency of RARRES-1 promoter methylation and its potential roles in the biologic behavior of this tumor.

**Design:** A total of 136 cases of HNSCC were included in this study. Complete clinical follow-up is available for all 136 HNSCC patients. DNA samples from these tumors were extracted, modified with sodium bisulfite, followed by methylation-specific PCR (MSP) using gene-specific and methylation-specific primer sets for RARRES-1 gene.

**Results:** Aberrant promoter methylation of RARRES-1 gene was detected in 59 of 136 (43%) cases of HNSCC. The findings were then correlated with various pathologic and clinical parameters. The presence of RARRES-1 promoter methylation in HNSCC is not correlated with tumor size, nodal status, clinical stage and 5-year survival. The pattern of RARRES-1 promoter methylation was also compared with patterns of promoter methylation at hMLH1, MGMT and p16 genes that were previously characterized in these tumors. There is a statistically very significant correlation between RARRES-1 and p16 gene in term of pattern of promoter hypermethylation ($p < 0.001$).

**Conclusions:** Aberrant promoter methylation of RARRES-1 gene occurs frequently in invasive head and neck cancer (43%). In light of p16 gene as the earliest altered gene established so far in head and neck squamous carcinogenesis and a paralleled promoter methylation pattern between p16 and RARRES-1 gene, the RARRES-1 promoter methylation may represent another early marker for head and neck squamous carcinogenesis.

### 1118 HPV In Situ Hybridization Analysis of Laryngeal Squamous Papillomatosis: A Comparison of Juvenile-Onset Versus Adult-Onset

*S Zafar, R Garcia, H Yee, Y Huan, YH Wen, B Wang.* New York University School of Medicine, New York, NY; Mount Sinai Medical Center, New York, NY.

**Background:** Laryngeal squamous papillomas are benign neoplasms, often affecting the true vocal cords. In childhood, they can be multiple with local recurrences, and are often self-limited. In adults, the rate of malignant transformation of these lesions varies between 1.6% and 4%. Papillomas have been shown to be associated with Human papilloma virus (HPV). We performed HPV in situ hybridization on 10 patients with laryngeal papillomatosis.

**Design:** Eighteen excisional biopsy specimens of the larynx were obtained from 10 patients over a four year period (2003-2007) from 2 tertiary institutions. Patients ranged in age from 4 months to 78 years (average, 39 years), divided into juvenile-onset group (<18 years of age), and adult onset (>18 years of age). Automated HPV in situ hybridization (ISH) analysis was performed on 4µm thick formalin fixed and paraffin embedded tissue sections, using a Bench Mark XT (Ventana Medical Systems, Tucson, AZ) with DNA probes for low risk HPV (types 6,11) and high risk HPV (types 16,18,31,33,35,39,51,52,56,58 and 66). Positive and negative controls were hybridized alongside the study cases. Positive staining was visualized using the precipitating chromogenic reaction NBT/BCIP with a nuclear localization.

**Results:** Four of 4 patients in the juvenile-onset group (average age: 5 years) had recurrent papillomatosis. Two of the 4 patients (50%) demonstrated HPV-ISH positivity for low risk HPV. High risk HPV was not identified in the juvenile onset group. In the adult-onset group (average age: 61 years), 3 of 6 patients had recurrent disease. Three of 6 patients in this group were positive for low risk HPV and one patient was positive for high risk HPV. The patient with high risk HPV had mild dysplasia on the tissue section.

**Conclusions:** In children, the presence of low risk HPV may be associated with multiple recurrences and high risk HPV is not identified. In adults, laryngeal papillomatosis can be associated with both low and high risk HPV. In this group, high risk HPV may be a potential marker for pre-malignant change but more extensive studies are needed to further classify this observation. Furthermore, ISH provides histological correlation with viral detection.

# Hematopathology

### 1119 Myeloid-Associated Antigen Expression Is an Adverse Factor for Complete Remission Following Induction Chemotherapy of Adult Precursor T-Lymphoblastic Leukemia/Lymphoma (T-ALL)

*M Al-Khabori, S Samiee, S Fung, W Xu, J Brandwein, W Brien, B Patterson, H Chang.* University Health Network, and University of Toronto, Toronto, Canada.

**Background:** Prognostic studies of T-ALL have been in small series with conflicting results. We retrospectively reviewed our experience of adult T-ALL patients to identify clinical and pathologic prognostic factors and build a risk-stratification model for induction chemotherapy.

**Design:** Our study included 67 patients with precursor T-ALL diagnosed between 1990 and 2007. They were evaluated by morphology, flow cytometric immunophenotyping and karyotype analysis. The patients were treated according to our institutional acute leukemia protocols and their clinical data were reviewed.

**Results:** At presentation, the patients' median age was 30 years, 52 were male and 15 female, 72% had lymphadenopathy, 39% a mediastinal mass, 24% CNS involvement and 25% splenomegaly. The median initial WBC was 21.3 x $10^9$/L (range 0.10-510.0). Blasts expressed CD34 in 46% of cases, CD10 in 33% and at least one myeloid-associated antigen in 28%. Karyotypes were abnormal in 36% of cases. Fifty-six of 64 patients (87.5%) who underwent induction chemotherapy achieved complete remission (CR) on protocols including vinca alkaloids, anthracyclines and corticosteroids. On univariate analysis; age, gender, initial WBC, CD10, CD34 and abnormal karyotype did not predict CR but patients expressing at least one myeloid-associated antigen had a CR of 74% compared to 94% (P=0.04) for patients not expressing myeloid antigens. In particular, CD33 expression without CD13 predicted a worse response (CR of 50% vs. 92%, P=0.02). Twenty-four patients relapsed with a median relapse-free survival (RFS) of 41.6 months (95%CI: 21.6-55.9). The RFS was longer for patients with an initial WBC of 3-50 x $10^9$/L and for cases expressing CD10 but neither was statistically significant (P=0.17, P=0.12). Patients with CD10 expression had a longer median OS at 44.7 months (versus 19.2 months for CD10 negative patients) but again, the difference was not statistically significant (P=0.10). Age, gender, CD34, myeloid-associated antigen expression and karyotype did not influence RFS or OS.

**Conclusions:** Our study indicates that expression of myeloid-associated antigens, especially CD33 expression without CD13, is an adverse prognostic factor for complete remission of adult T-ALL and should be considered for induction chemotherapy risk-stratification.

### 1120 Mixed Lineage Kinase-3 (MLK3) Expression in Follicular Lymphoma

*S Almiski, N Razumilava, M Kurrer, E Levi.* Harper University Hospital, Wayne State University, Detroit; Cantonal Hospital Aarau, Aarau, Switzerland; John D. Dingell VAMC, Detroit.

**Background:** Mixed lineage Kinase-3 (MLK3) is a protein kinase that is part of the MAPK signaling system which plays a role in the activation of the JNK and ERK pathways. MLK3 gene was shown to be overexpressed in follicular lymphomas in a gene array study. We had also shown MLK3 to be a poor prognostic factor in diffuse large B-cell lymphomas. In this study we investigated the role of MLK3 on survival in follicular lymphomas.

**Design:** Patients with follicular lymphoma (n=176), which were followed up at University Hospital, Zurich were selected to generate a tissue microarray. Patient median follow up was 9.4 years. Immunohistochemical staining was performed utilizing Santa Cruz antibody (MLK3, C20, sc-536). In addition, CXCR4, Fak, and CARP-1 stains were performed and correlated with MLK3 expression. The cases were scored according to staining intensity and percentage tumor cell staining. We scored the staining by multiplying staining intensity with percent cells staining. A cutoff value of 100 was utilized to classify the cases as expressors vs. non-expressors.

**Results:** MLK3 expression on normal tonsil was minimal to negligible. MLK3 staining was present in 105/176 cases of follicular lymphoma. The staining was predominantly cytoplasmic. MLK3 expression did not have any correlation with overall survival in the follicular lymphomas tested. We also analyzed a transformed subgroup of diffuse large cell lymphomas of follicular origin and found that expression of MLK3 was a poor