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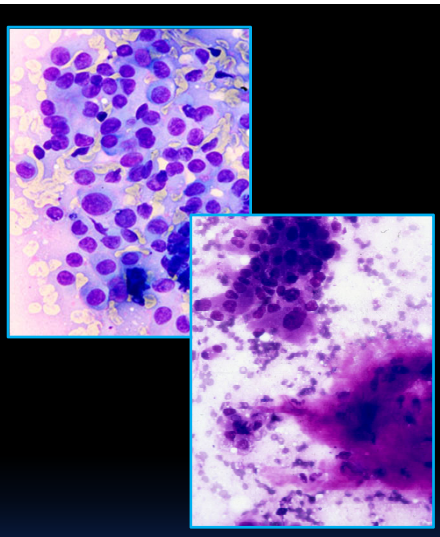
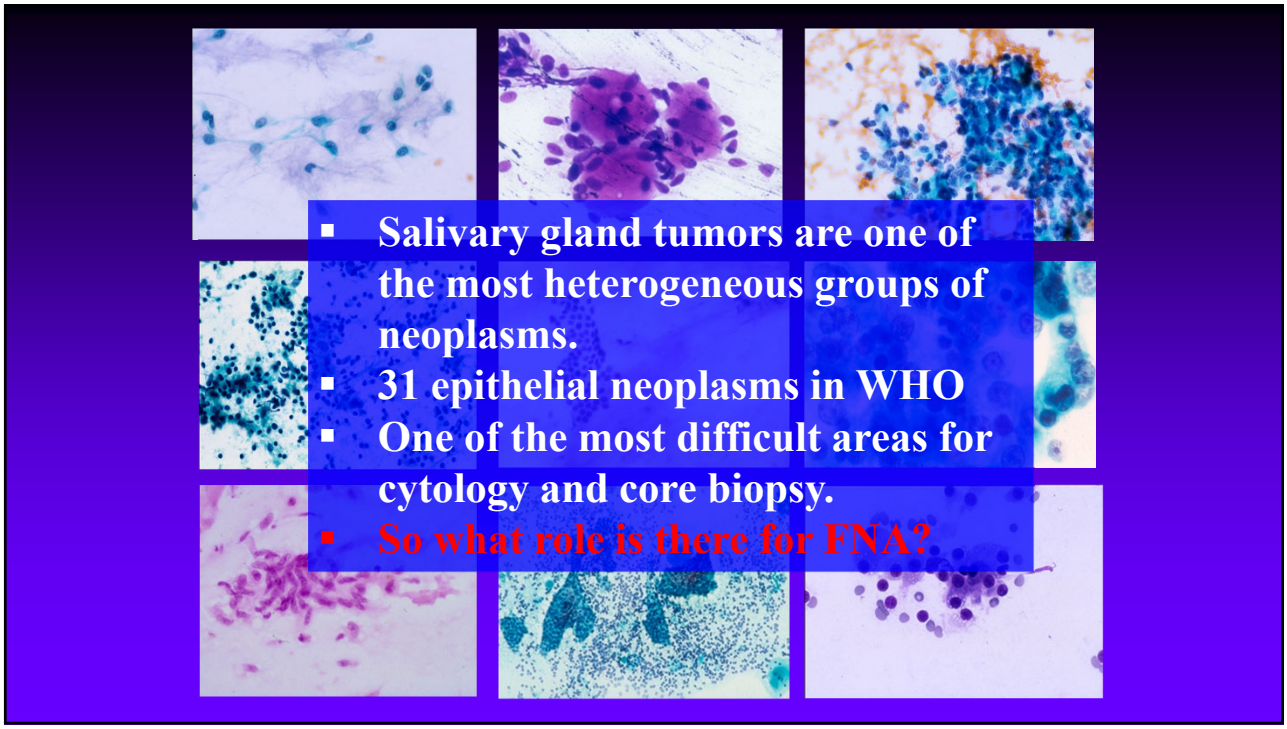


Salivary Gland Cytology:
Combining Cytomorphology and Ancillary Studies

Disclosure of Relevant Financial Relationships

No financial or other conflicts to disclose.

Information presented includes work by colleagues & collaborators at the MGH as well as work from groups around the globe.



- Salivary gland tumors are one of the most heterogeneous groups of neoplasms.
- 31 epithelial neoplasms in WHO
- One of the most difficult areas for cytology and core biopsy.
- So what role is there for FNA?

FNA vs Core Biopsy

Major limitation is inability to assess for invasion

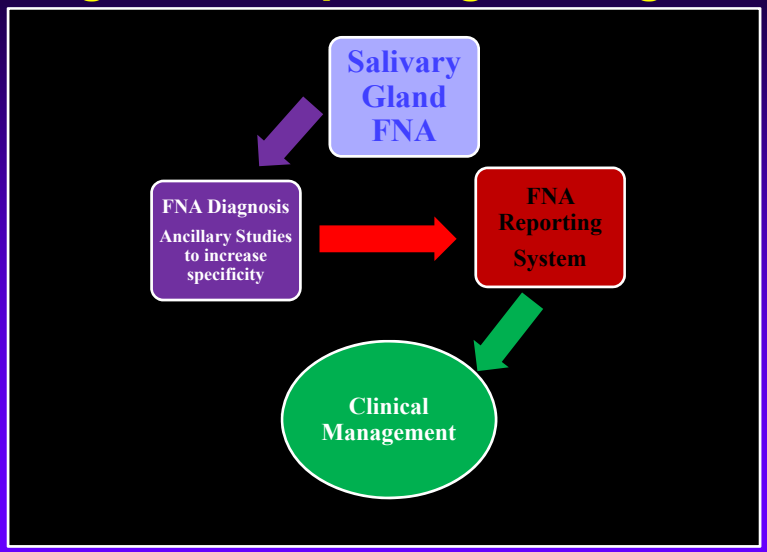
Preop Dx has significant implications for management!

FNA	Core Biopsy
• Faster TAT; ROSE 1 hr	• 1-2 days
• Multiple FNA sampling	• Limited sampling
• Complications are rare	• Complications are uncommon
• No risk of needle track seeding or nerve damage	• Needle track seeding & nerve injusry are considerations
• Material for ancillary studies may be limited	• Better source for ancillary studies
• Used for major SG lesions at MGH	• Used primarily for minor SG lesions at MGH

Reporting System for Salivary Gland FNA

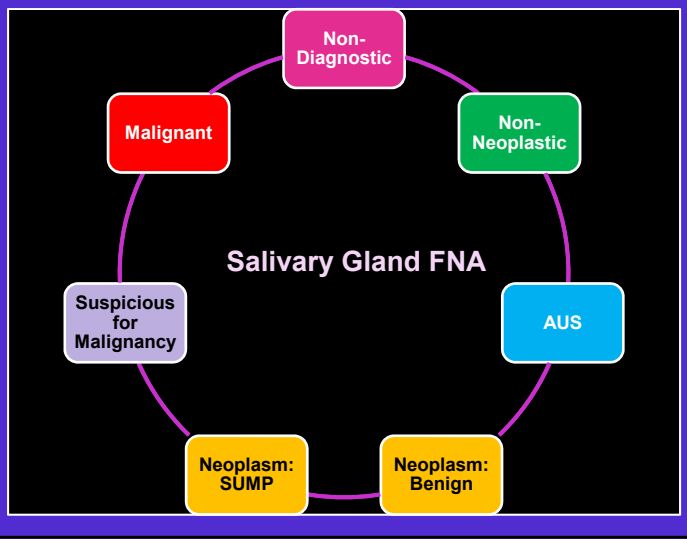
Why do we need a new reporting system for salivary gland cytology?

Salivary Gland FNA: Diagnosis, Reporting, Management



The Milan System for Reporting Salivary Gland Cytopathology (2018 & 2024):

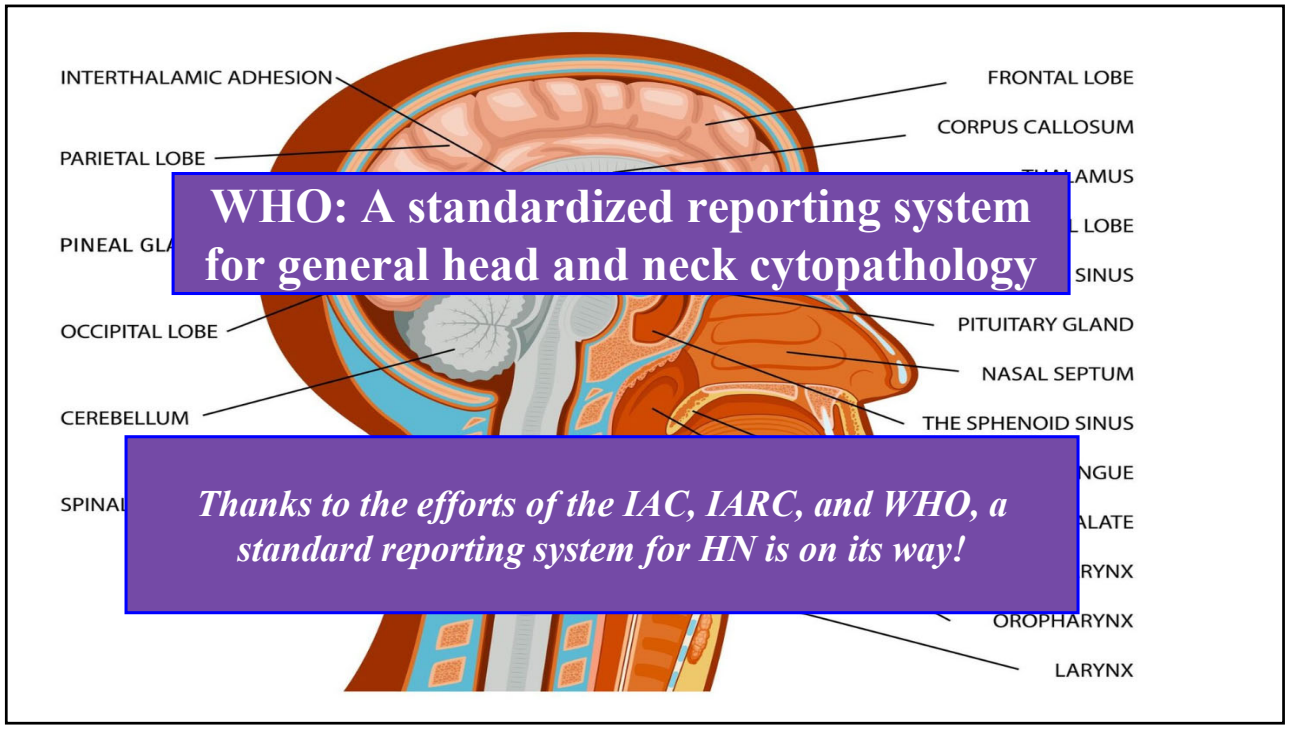
Diagnostic Categories



The Milan system for reporting salivary gland cytopathology: A comprehensive review of the literature

Jalal B. Jalaly MBBS, MS[1] | Sahar J. Farahani MD, MPH[2] | Zubair W. Baloch MD, PhD[1]

- **Calculated ROM's in Second Edition:**
  - Non-Diagnostic 15%
  - Non-Neoplastic 11%
  - AUS 30%
  - Neoplasm: Benign <3%
  - Neoplasm: SUMP 35%
  - Suspicious 83%
  - Malignant >98%



The WHO Reporting System for Head and Neck Cytopathology

- **6 Diagnostic Categories:**
  - **Insufficient/Inadequate/Non-Diagnostic**

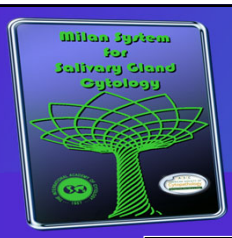
ROMs for each diagnostic category are being estimated based upon existing published data.

  - **Neoplasm of Uncertain Malignant Potential (NUMP)**
  - **Suspicious for Malignancy**
  - **Malignant**

Six-Tiered Systems for HN, SG, Thyroid

The WHO Reporting System for general head and neck entities can be applied to both salivary and thyroid gland FNABs.

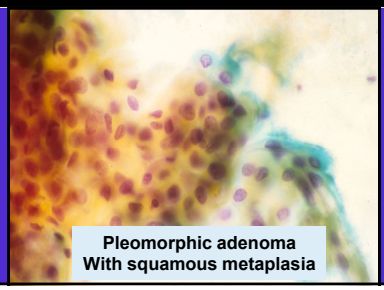
WHO HN Cyto	Milan	Bethesda Thyroid
•Non-Diagnostic	•Non-Diagnostic	•Non-Diagnostic
•Benign	•Non-Neoplastic	•Benign
•Atypical	•AUS	•AUS
•NUMP	•Neoplasm/SUMP	•Follicular Neoplasm
•Suspicious for malignancy	•Suspicious for malignancy	•Suspicious for malignancy
•Malignant	•Malignant	•Malignant

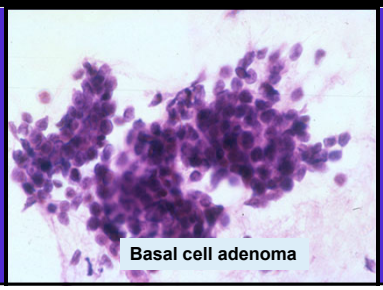


Neoplasm: UMP

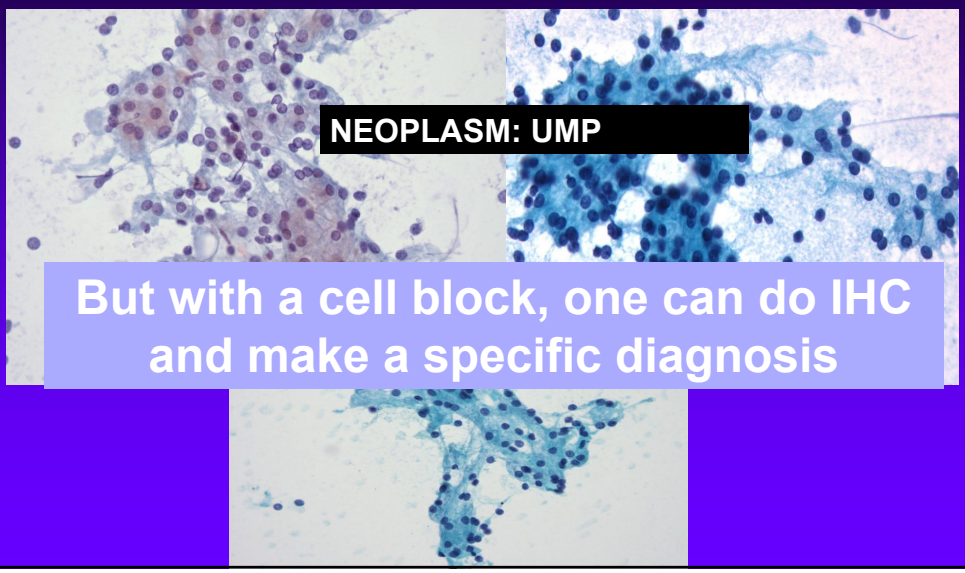
ii) Salivary Gland Neoplasm of Uncertain Malignant Potential:

- Diagnostic of a neoplasm; however, a diagnosis of a specific entity cannot be made.
- A malignant neoplasm cannot be excluded.
- ROM is 35%
- Many benign neoplasms and some low-grade carcinomas


Pleomorphic adenoma
With squamous metaplasia


Basal cell adenoma

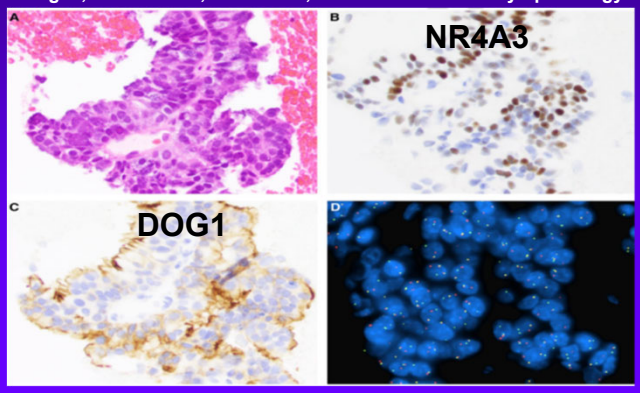
55 year-old woman with a 2 cm right parotid mass



But with a cell block, one can do IHC and make a specific diagnosis

Acinic Cell Carcinoma: Cell Block - DOG1+, SOX10+, NR4A3+

JM Skaugen, RR Seethala, SI Chiosea, MS Landau. Cancer Cytopathology 2020



For FNA to be competitive as a diagnostic test, we strive to shift cases from UMP (Indeterminate) to the Malignant or Benign categories.

Salivary Gland FNA and Ancillary Markers

Salivary Gland FNA: New Markers and New Opportunities for Improved Diagnosis

Marc P. Pusztaszeri, MD[1]; Joaquin J. García, MD[2]; and William C. Faquin, MD, PhD[3,4]

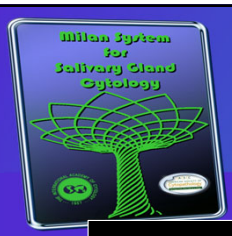
- **Obtaining material for ancillary studies is key to improving the accuracy of salivary gland FNA**
- **Used judiciously on a case-by-case basis**
- **Cell blocks are preferred for IHC and molecular studies.**

Increasing Availability of Molecular Markers For Salivary Gland Tumors

- Secretory carcinoma:
  - ETV6-NTRK3; t(12:15)
- Pleomorphic adenoma & Ca ex PA:
  - PLAG1; t(3;8)
  - HMGA2 rearrangement
- Clear cell carcinoma:
  - EWSR1-ATF1; t(12:22)
- Mucoepidermoid carcinoma:
  - MEGT1/MAML2; t(11;19)

FISH is good for a specific entity
Multiplex PCR is best for a DDX

- Adenoid cystic carcinoma:
  - MYB-NFIB; t(6:9)
- Basal cell adenoma:
  - CTNNB1 mutation
- Acinic cell carcinoma
  - NR4A3; t(4:9)
- Epithelial-myoepithelial carcinoma
  - RAS mutation
- Intraductal carcinoma
  - NCOA4-RET



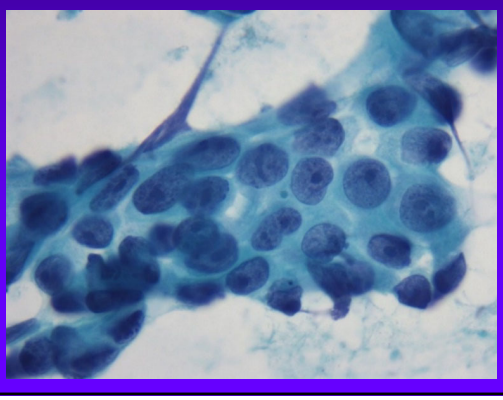
Malignant

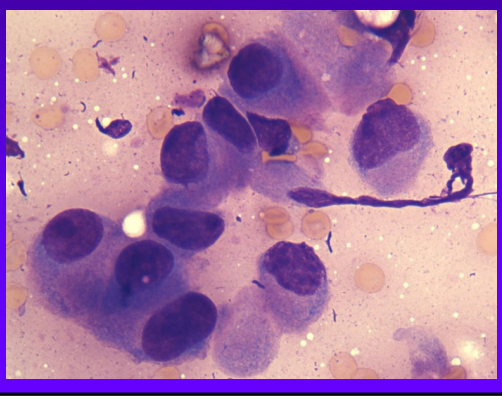
- Aspirates which are diagnostic of malignancy.
- Sub-classify into specific types and grades of carcinoma
  - –Grading is critical for clinical management
- "Other" malignancies: lymphomas, sarcomas, metastases from skin
- ROM > 98%

MALIGNANT CATEGORY

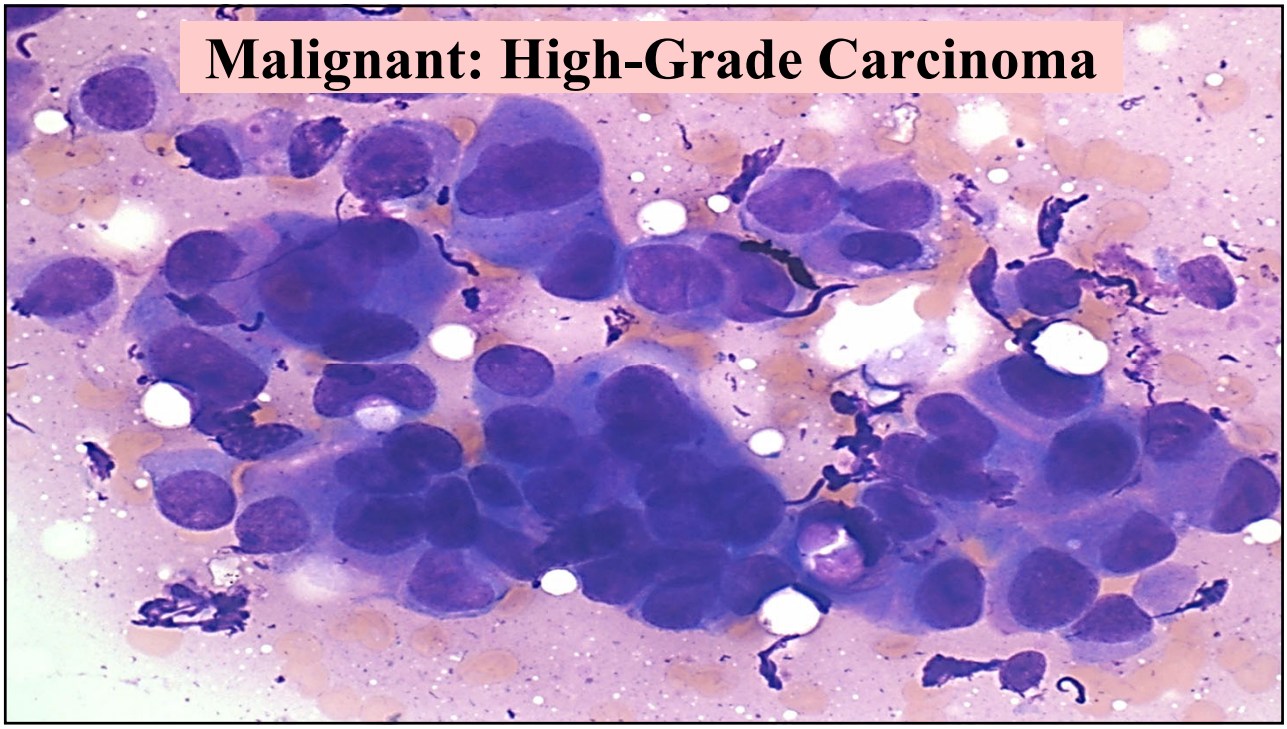
- 1) Classic cytologic features of a particular SG cancer (includes selected low-grade cancers)
- 2) Overt malignant features (high-grade cancers)
- 3) Ancillary studies are diagnostic of cancer

An 80 year-old man presents with right facial paresthesia, and a 3 cm right parotid mass. An FNA of the right parotid mass was performed under U/S guidance in the FNA clinic.



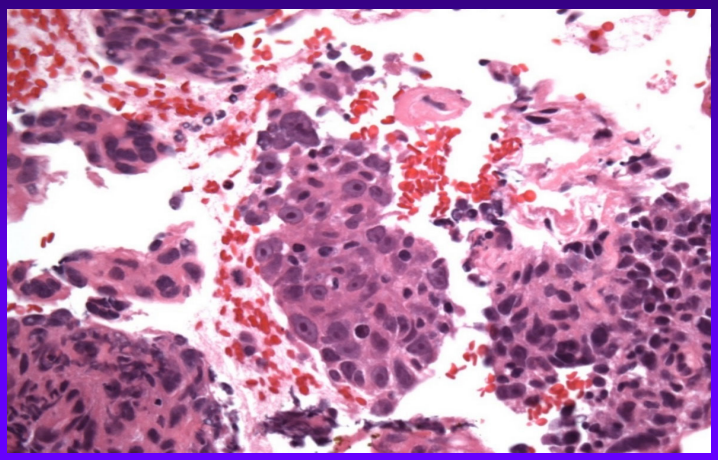


Malignant: High-Grade Carcinoma



CELL BLOCK

• IHC on cell block shows that the carcinoma is positive for GATA-3, androgen receptor, and 3+ Her2 = SALIVARY DUCT CARCINOMA

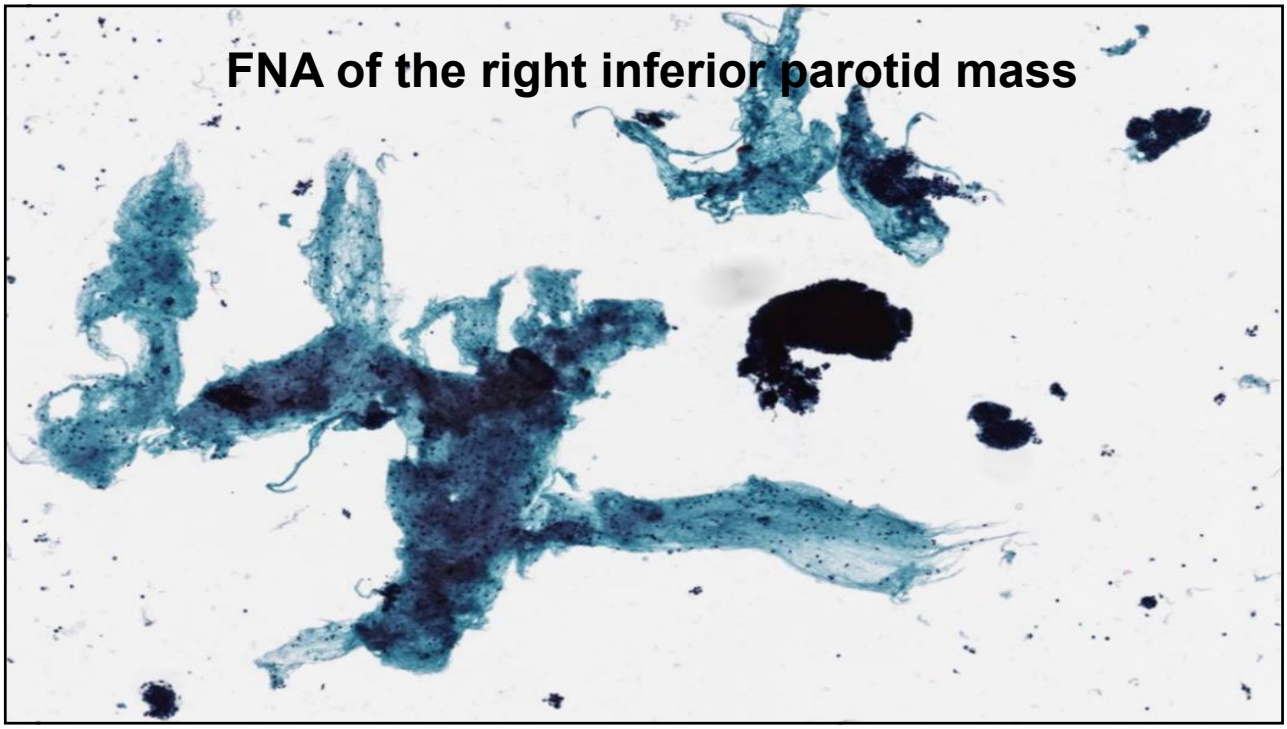


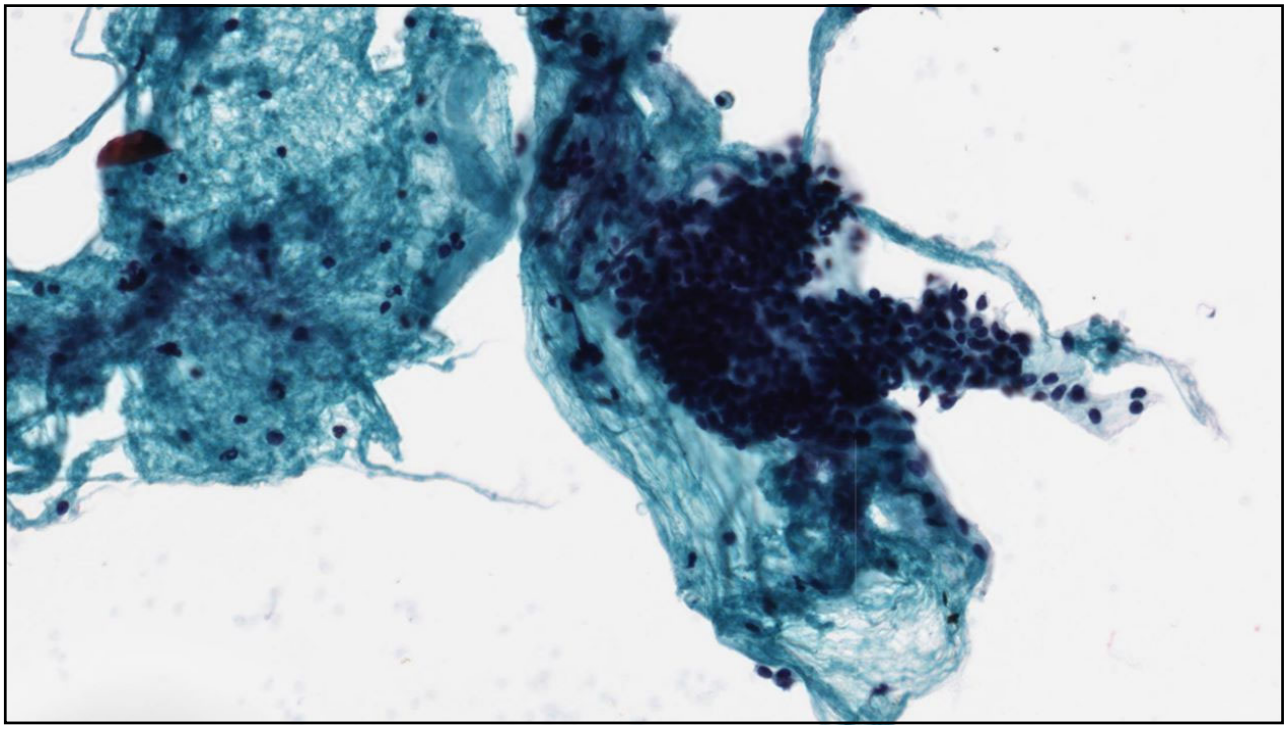
Salivary Gland FNA Case

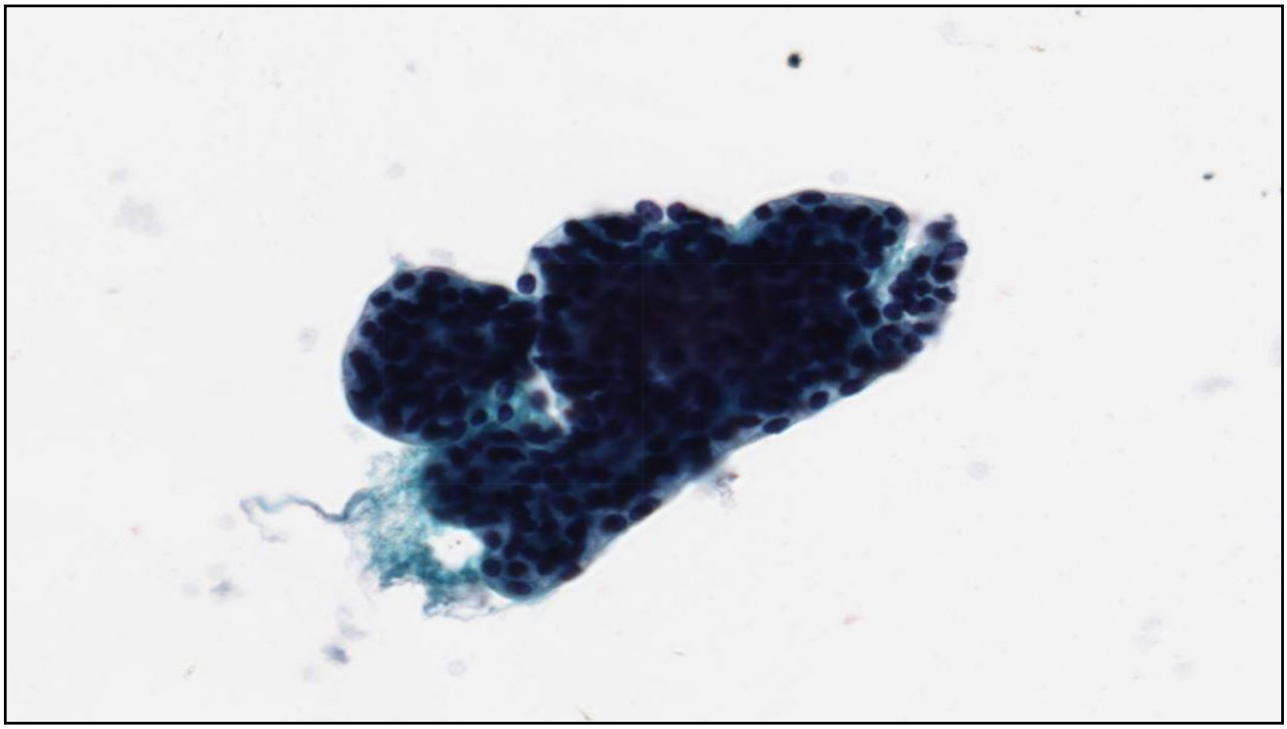
A 38 year-old woman with a slowly enlarging, palpable 1.5 cm right neck mass medial to the angle of the jaw.

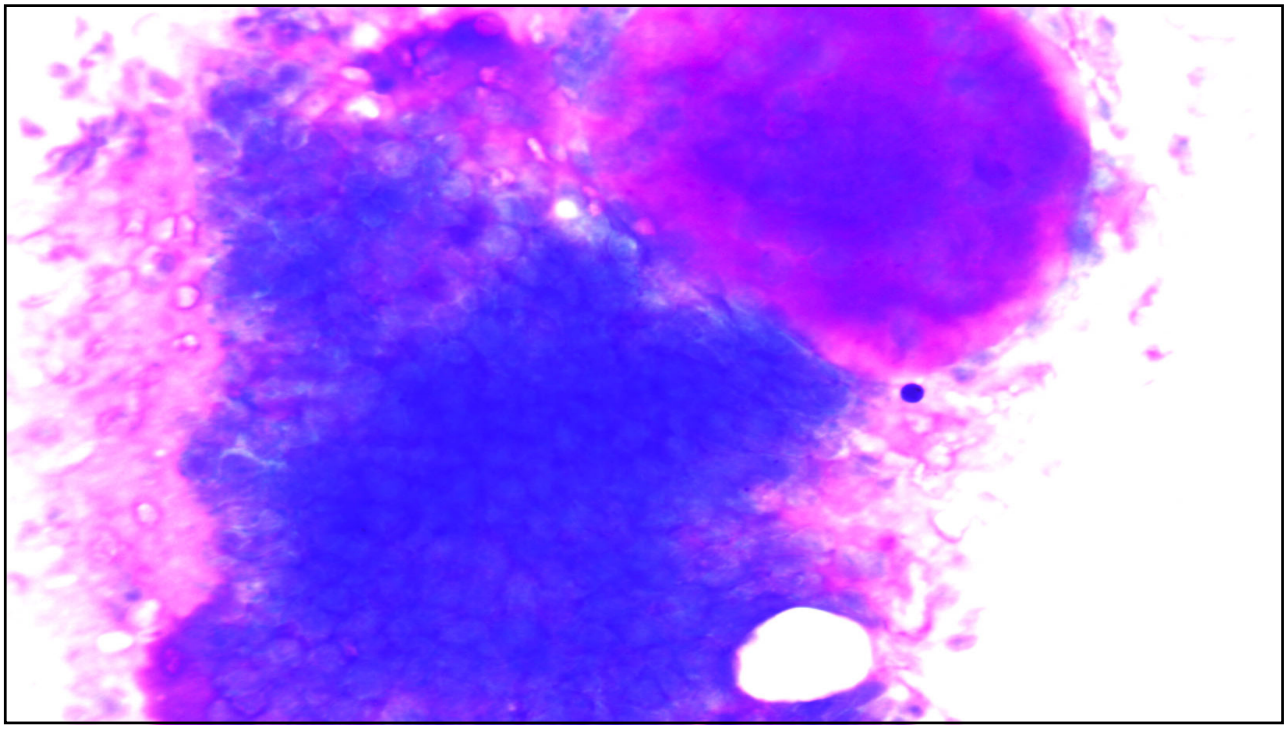
MRI:
1.5 cm enhancing mass of right parotid
DDX: LN, Pleomorphic adenoma, other tumors

Axial T2-weighted image


FNA of the right inferior parotid mass







Primary FNA Differential Diagnosis of Basaloid Salivary Gland Neoplasms

- Basal cell adenoma/adenocarcinoma
- Cellular pleomorphic adenoma
- Adenoid cystic carcinoma

Cytologic Diagnosis:

NEOPLASM: UMP

Basaloid neoplasm. See note.

VS

Cytologic Diagnosis:

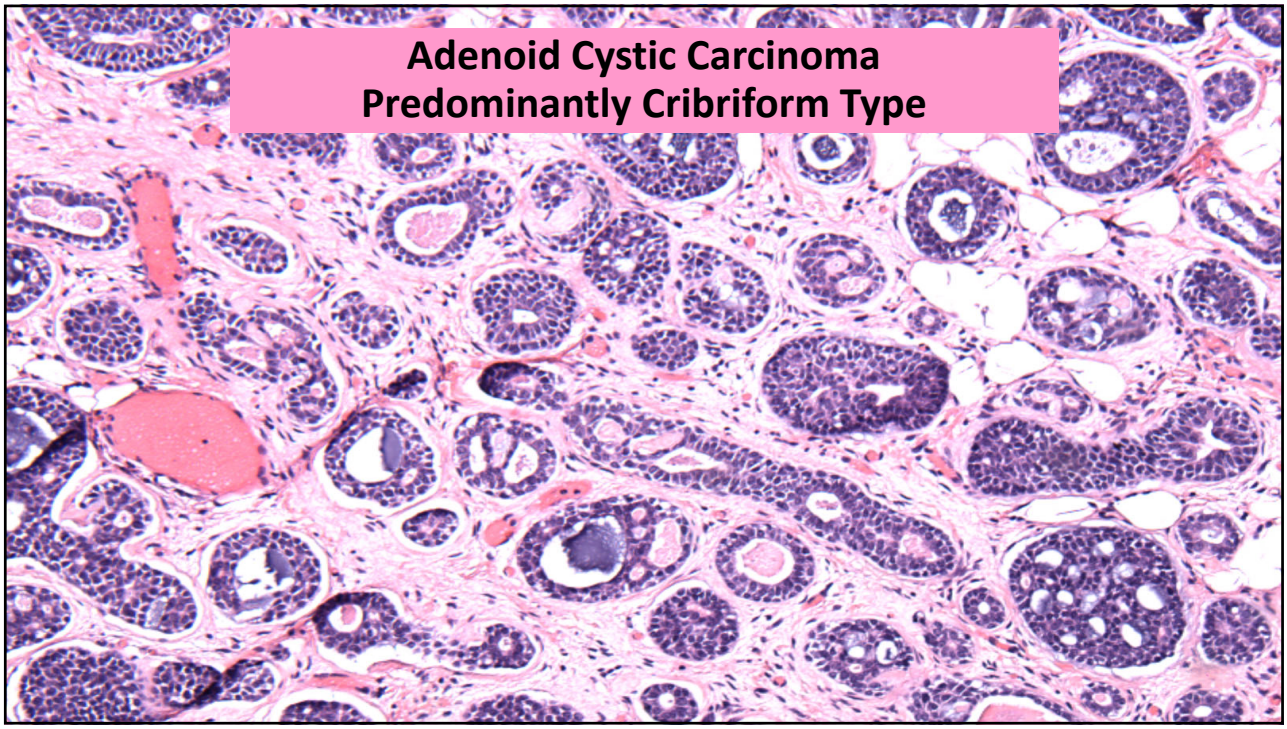
SUSPICIOUS FOR MALIGNANCY

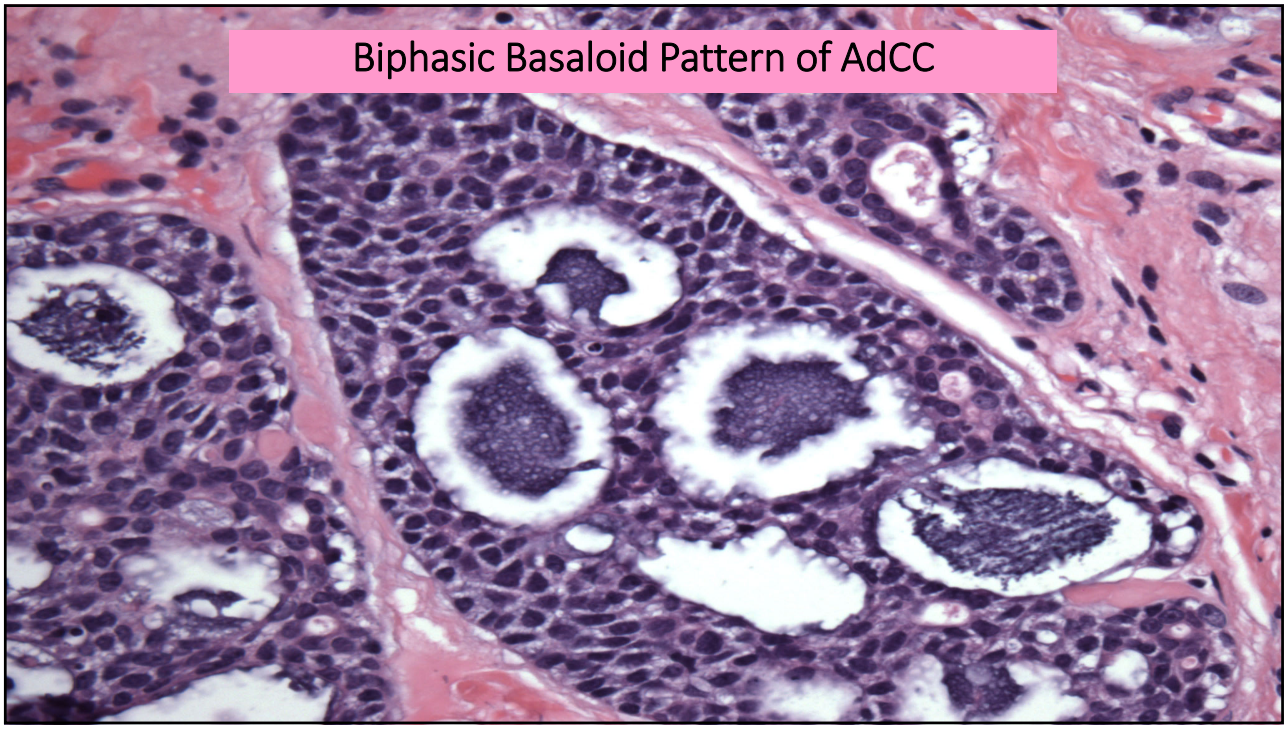
Basaloid neoplasm, highly suspicious for adenoid cystic carcinoma.

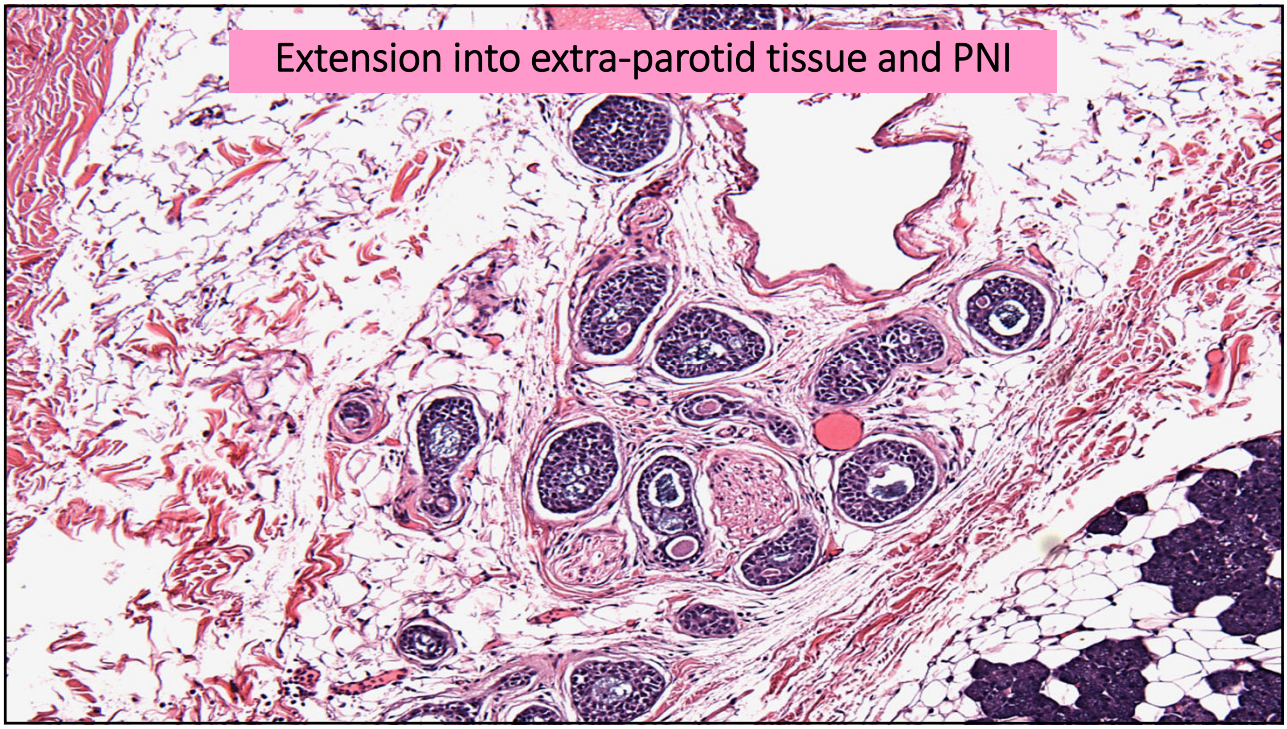
A repeat FNA was done for molecular testing – MYB fusion was detected by multiplex PCR from CB material confirming adenoid cystic carcinoma.

Clinical Management

The tumor was surgically excised:
Superficial and deep lobe parotidectomy.
Confirmed to be AdCC by frozen section; sacrifice of lower division of facial nerve and greater auricular nerve graft.
All frozen section margins were negative.


Adenoid Cystic Carcinoma
Predominantly Cribriform Type


Biphasic Basaloid Pattern of AdCC

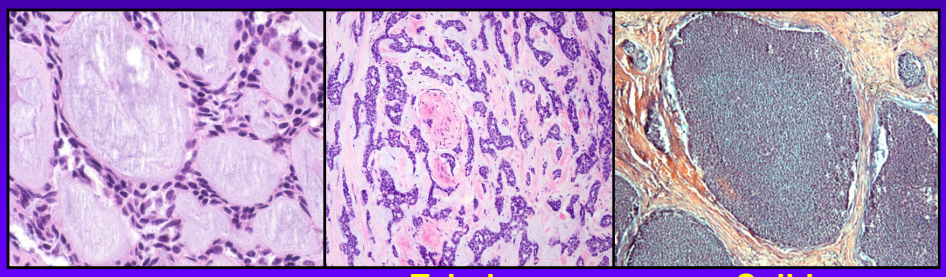


Adenoid Cystic Carcinoma

- Second most common salivary gland malignancy
  - 1200 new cases per year in the USA
  - 4-10% of all salivary gland neoplasms
- Median age 57 years old
- Major salivary glands (66%), Minor glands of oral cavity, sinonasal cavity, other anatomic sites (33%)
- Initial indolent behavior but poor long-term survival
  - 10 year survival of 40-60%; worse prognosis for solid type
  - Metastasis in >50%, especially to lung

Adenoid Cystic Carcinoma: 3 Histologic Patterns

- Three histologic patterns:
  - Cribriform
  - Tubular
  - Solid (20-30%)



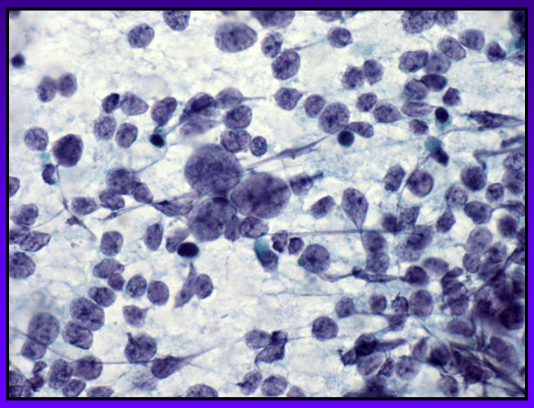
Cribriform | Tubular | Solid

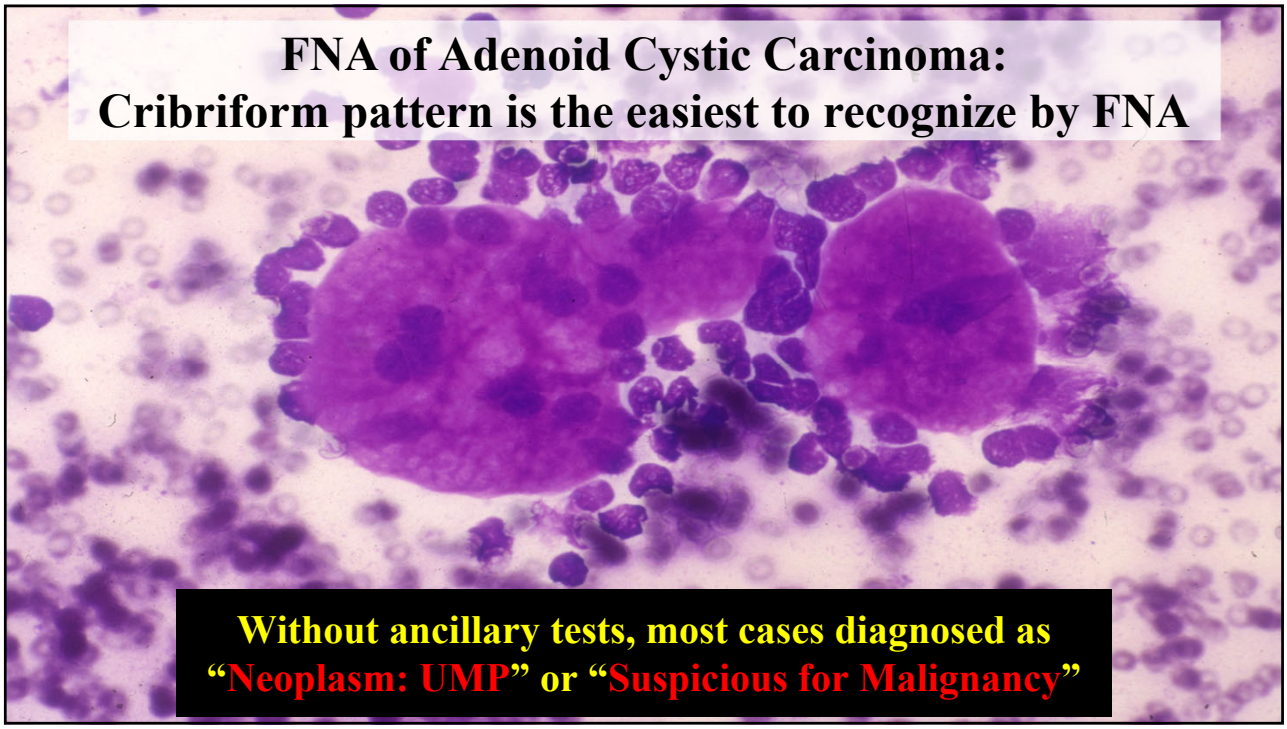
AdCC With High-Grade Transformation: A Highly Aggressive Cancer

Fine needle aspiration of salivary gland carcinoma with high-grade transformation: A multi-institutional study of 22 cases and review of the literature

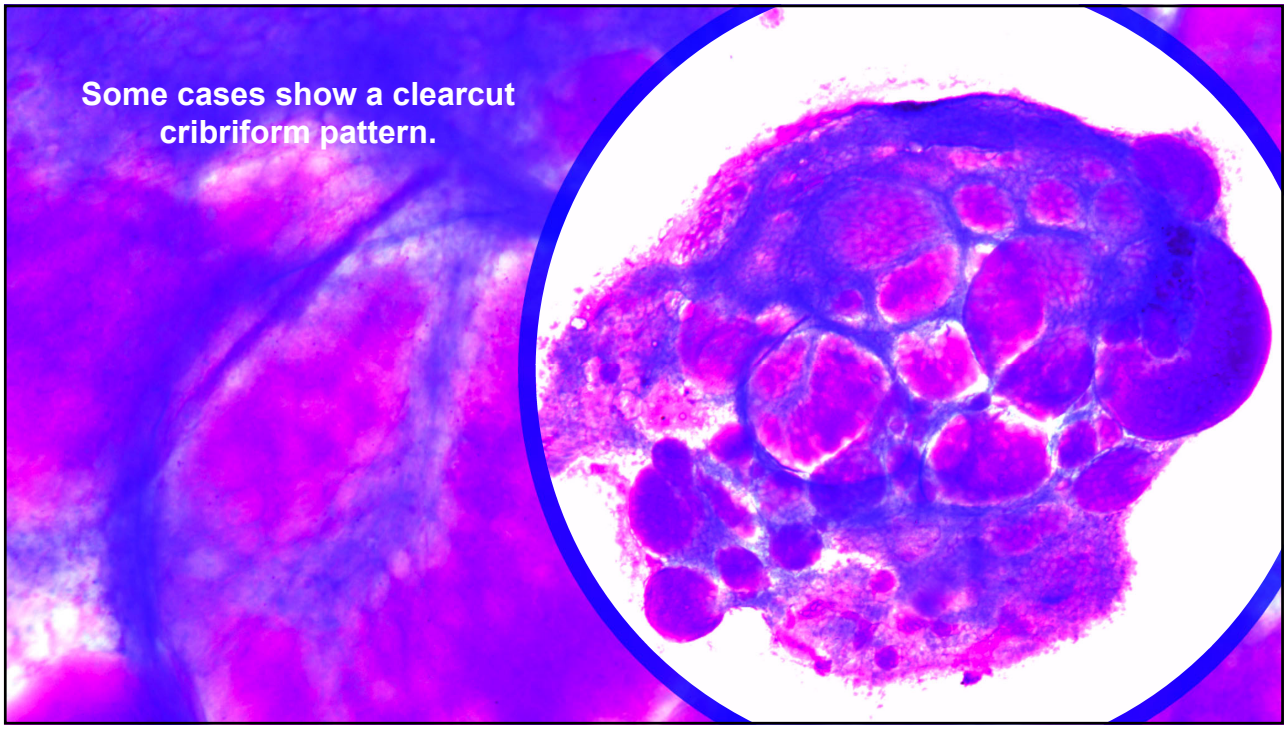
M Nakaguro, WC Faquin, ZW. Baloch, RL. Cantley, ML. Compton, KA. Ely, B Holmes; R Hu, DA. Kerr, K Montone, M Nishino, L Pantanowitz, ED Rossi, PM Sadow. Cancer Cytopathol, 2020.

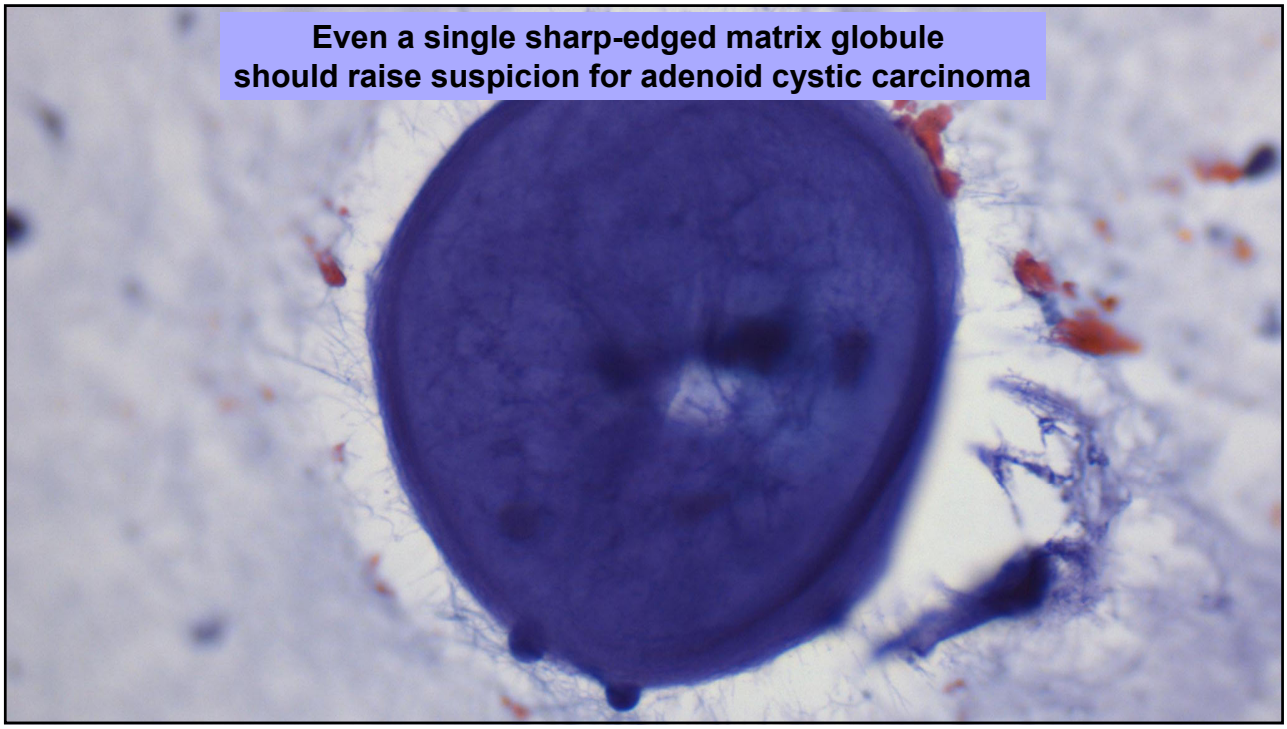
- Very aggressive clinical course
- FNA = Malignant
- Can occur in many different types of salivary gland carcinoma
  - Adenoid cystic carcinoma
  - Acinic cell carcinoma
  - Secretory carcinoma
  - Epithelial-myoepithelial carcinoma

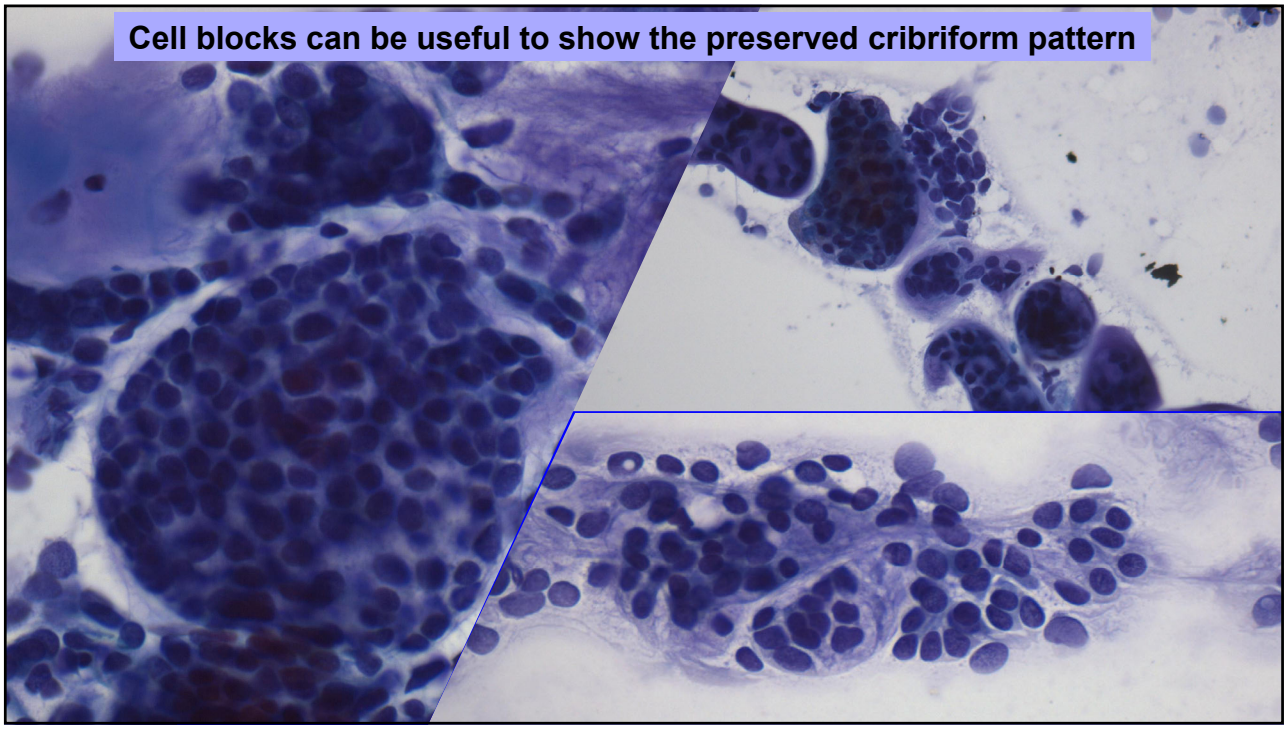




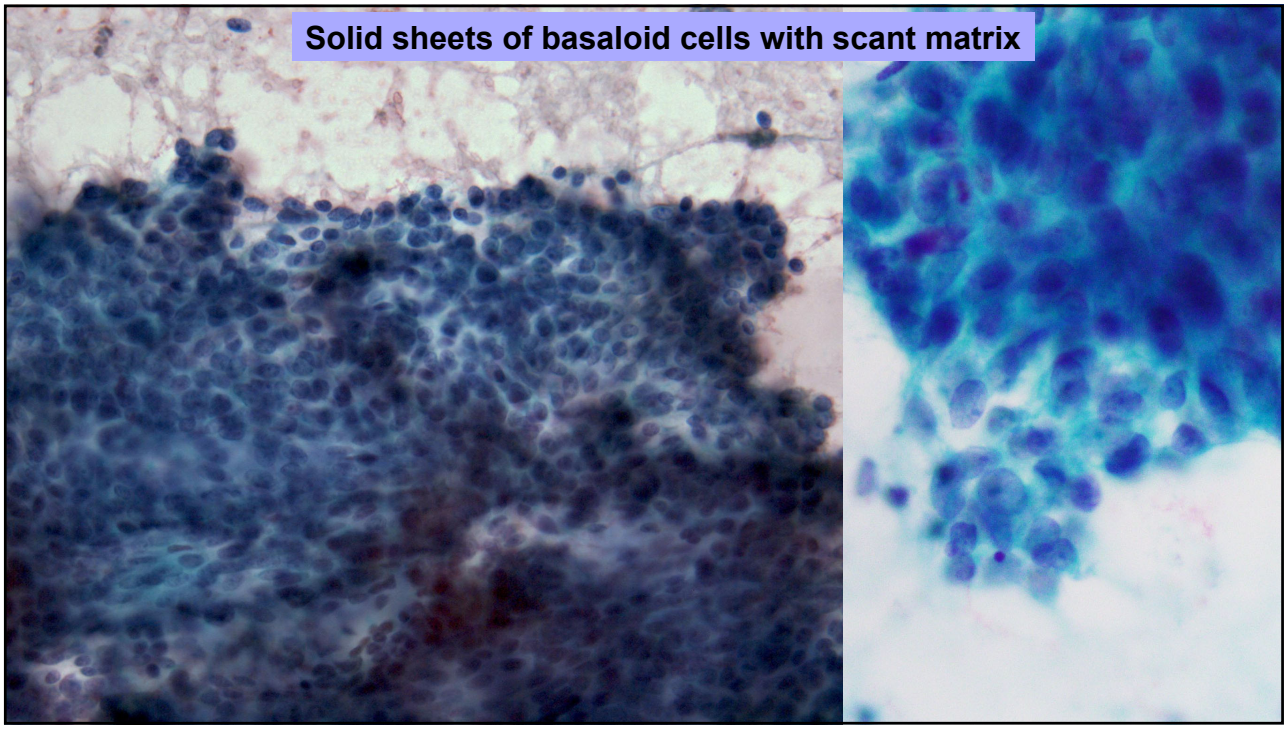
More images of classic cribriform adenoid cystic carcinoma

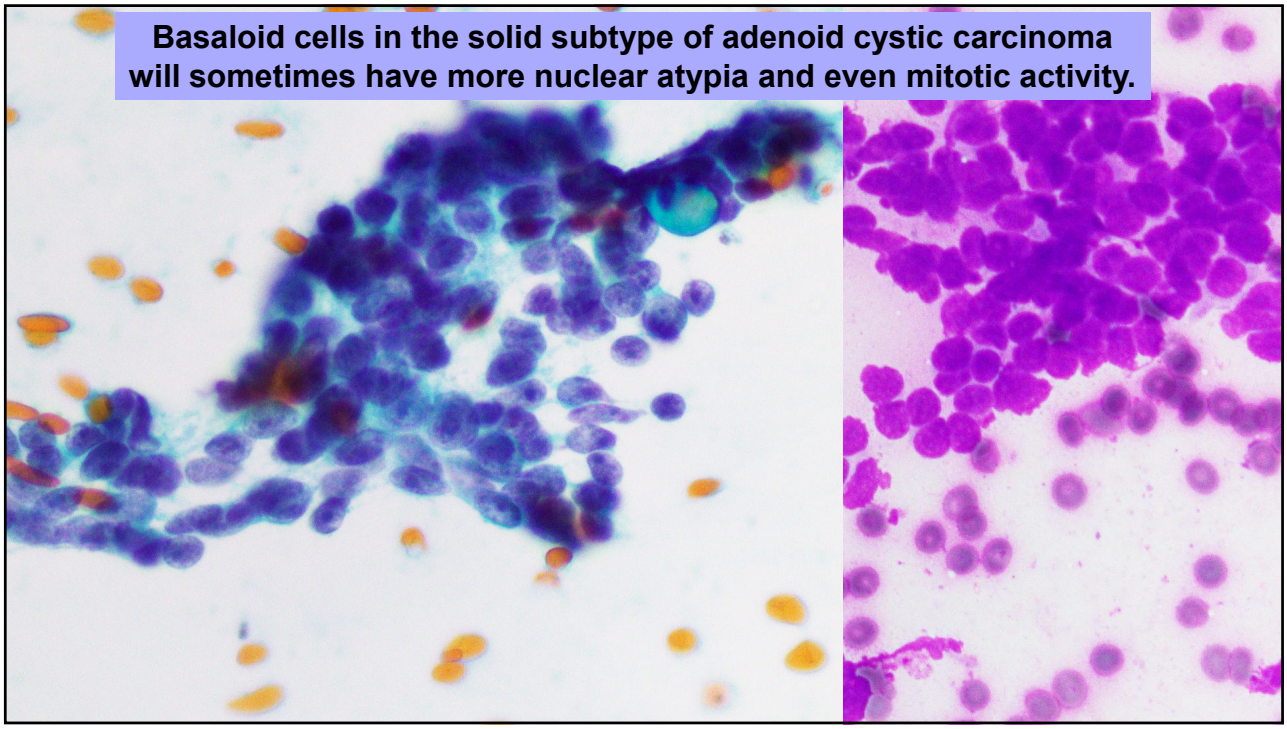

Some cases show a clearcut
cribriform pattern.


Even a single sharp-edged matrix globule
should raise suspicion for adenoid cystic carcinoma



Images of solid adenoid cystic carcinoma


Solid sheets of basaloid cells with scant matrix


Basaloid cells in the solid subtype of adenoid cystic carcinoma
will sometimes have more nuclear atypia and even mitotic activity.

Adenoid Cystic Carcinoma:

IHC can be helpful but is NOT specific!

Immunohistochemistry:

- Positive for keratin 7, CEA, EMA
- Positive for myoepithelial markers:
  - Smooth muscle actin
  - Calponin
  - S-100
  - Keratin 5/6
  - P63
- SOX10+
- CD117 (KIT) +
- MYB +
- NOTCH+

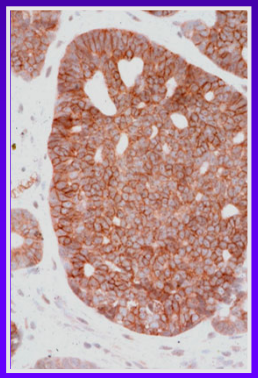
Adenoid Cystic Carcinoma and CD117 (KIT)

Expression of KIT (CD117) in Neoplasms of the Head and Neck: An Ancillary Marker for Adenoid Cystic Carcinoma

M. Mino, M.D., B.Z. Pilch, M.D., W.C. Faquin, M.D., Ph.D.
Department of Pathology, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts

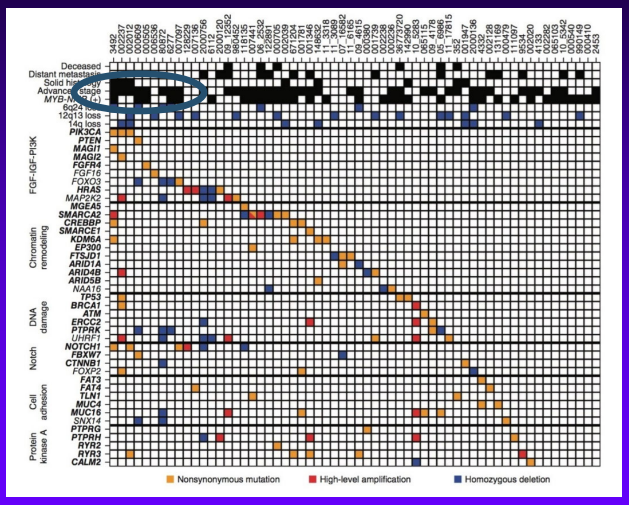
CD117 Immunohistochemistry:

- Over 90% are strongly positive for CD117 (KIT)
- Useful for all variants including solid form
- Protein overexpression but no mutation identified; no therapeutic role for imatinib as in AML and GIST.

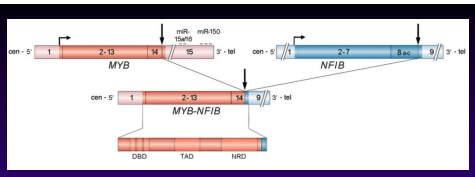


Mutational Landscape of Adenoid Cystic Carcinoma:

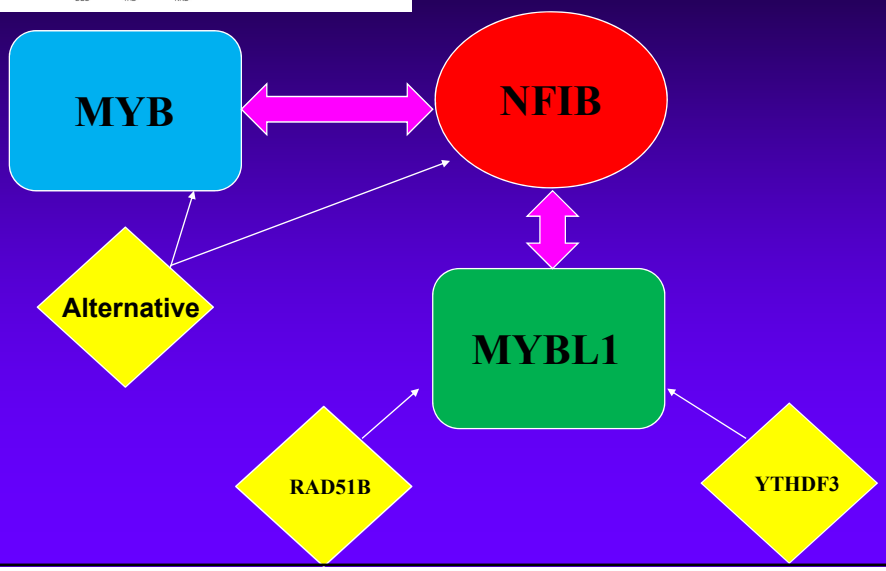
MYB Rearrangement is Most Common and has Been Associated with Poorer Prognosis in Some Studies



Ho et al, NatGen. 2013; 45(5): 791–800



MYB-NFIB fusion In AdCC



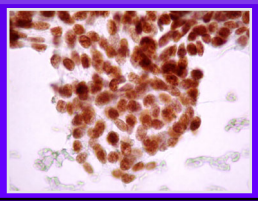
MYB Antibody and AdCC: *Diagnostics and Therapeutics!*

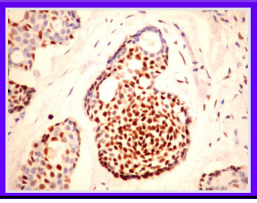
- Multiple MYB antibodies available
- Epitomics seems to work best for ETOH-fixed material
- >80% of AdCC are positive by IHC, but protein level expression is not specific
- Detection of MYB overexpression may be useful for identifying patients for targeted therapy

MYB immunostaining is a useful ancillary test for distinguishing adenoid cystic carcinoma from pleomorphic adenoma in FNAB specimens

Pusztaszeri M, Sadow M, Faquin W. Cancer Cytopath

FNA





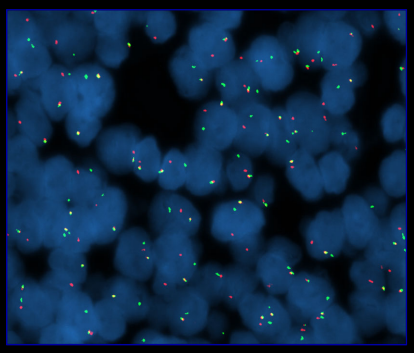
BIOPSY

Adenoid Cystic Carcinoma:

MYB Translocation Detection is DIAGNOSTIC

Cytogenetics:

- t(6:9) MYB oncogene-NFIB transcription factor
- In salivary gland, this finding by FISH is specific for AdCC

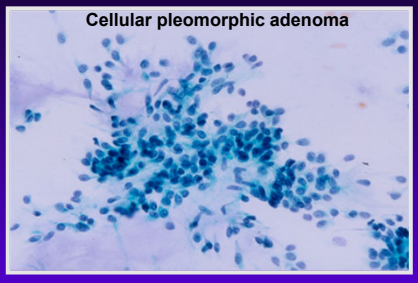


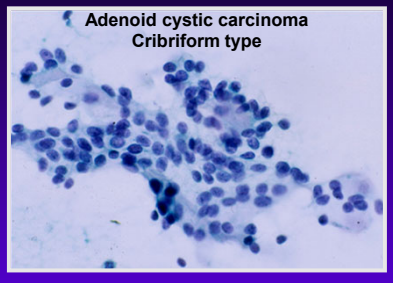
FISH contributed by Dr. Joaquin Garcia, Mayo Clinic

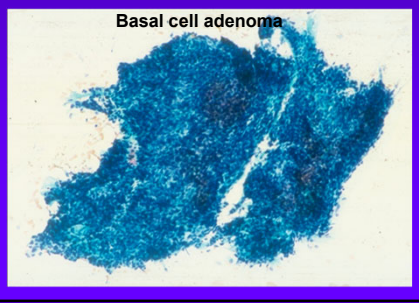
AdCC is a major problem for FNA!

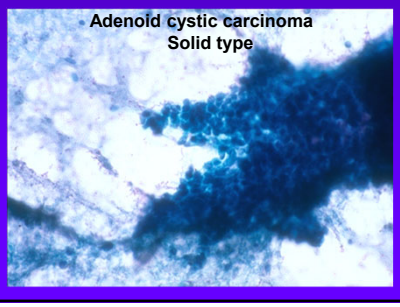
- Resembles other benign and malignant basaloid salivary gland tumors
- Significant clinical management implications
  - Radical resection
  - Radiation/Chemo and/or proton beam
- Usually requires ancillary studies for definitive FNA classification
  - Frozen section evaluation is an option

FNA Pitfall: Adenoid Cystic Carcinoma vs. Other Basaloid Neoplasms





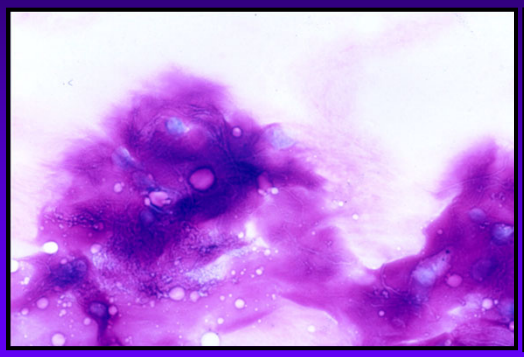




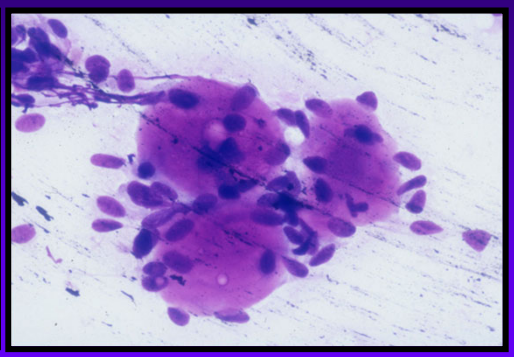
Pitfall: FNA Sample Preparation

Both alcohol-fixed and air-dried preparations are essential in the evaluation of matrix-containing tumors!

DQ stains help to highlight the distinguishing matrix qualities

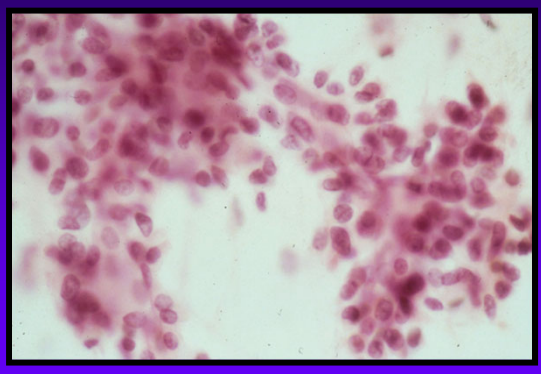


Pleomorphic adenoma

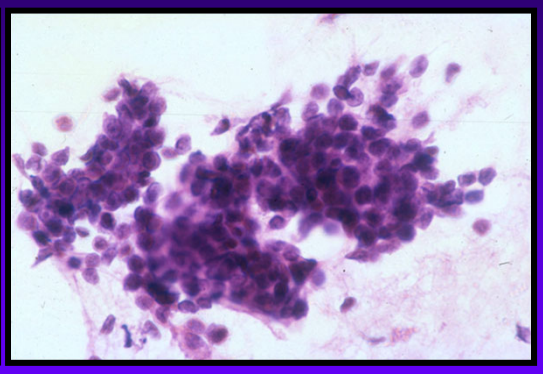


Adenoid cystic carcinoma

Beware: Solid Pattern of Adenoid Cystic Carcinoma vs. Cellular Pleomorphic Adenoma

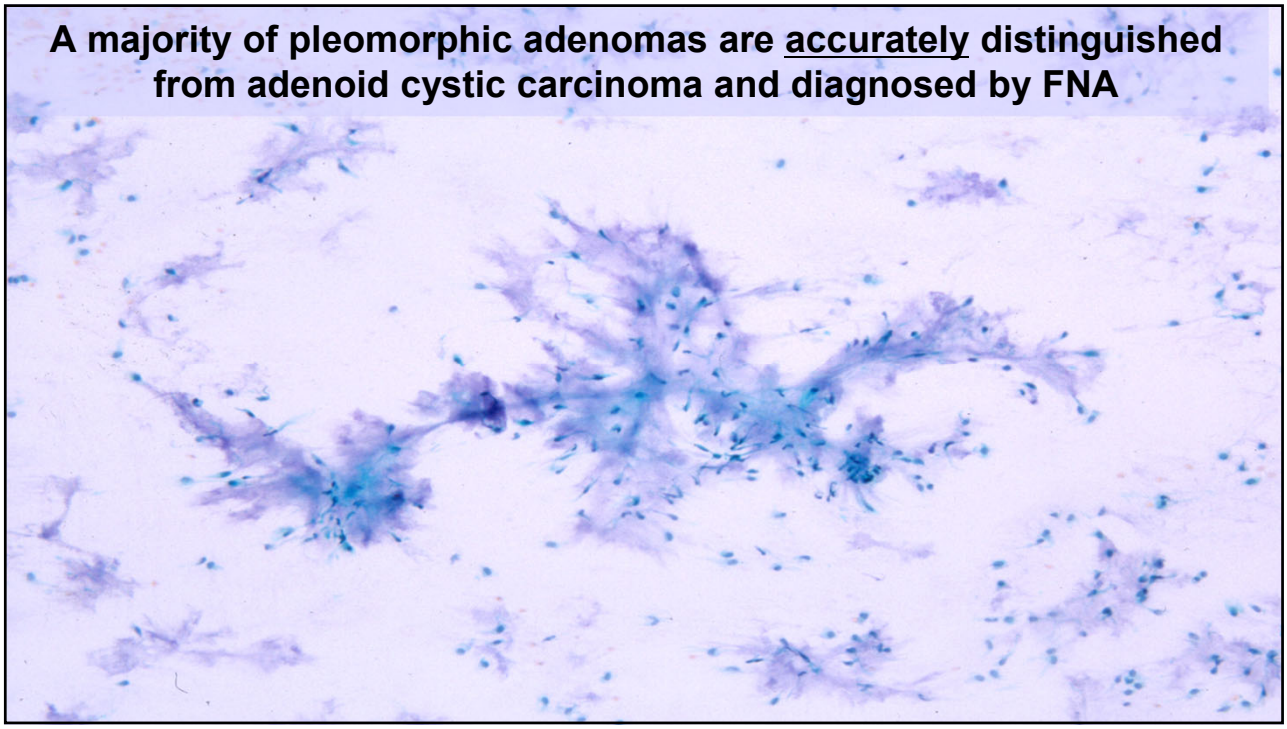


Pleomorphic adenoma

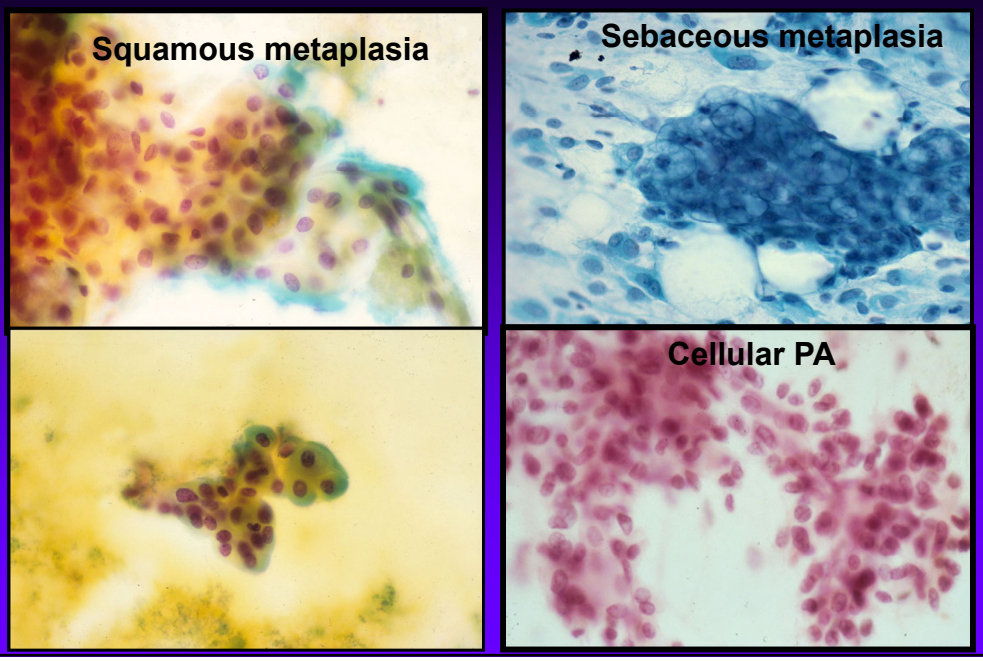


Adenoid cystic carcinoma

A majority of pleomorphic adenomas are accurately distinguished from adenoid cystic carcinoma and diagnosed by FNA



Pleomorphic adenoma: A small subset with cellular & metaplastic features are challenging



Pleomorphic Adenoma

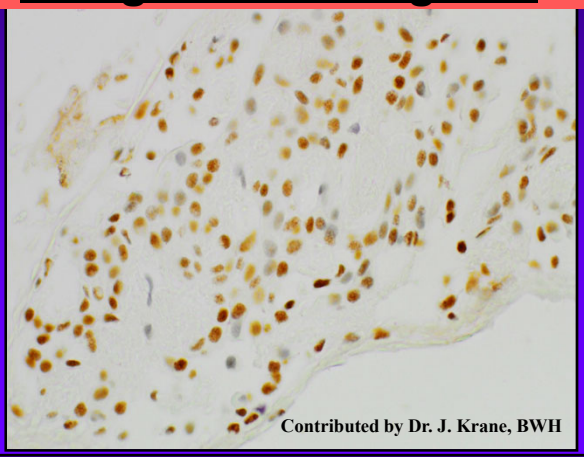
PLAG1 & HMGA2:

Two Very Useful Immunohistochemical Markers to Distinguish PA from Adenoid Cystic Carcinoma, Especially for Cell Blocks and Core Biopsies

PLAG-1 Immunoreactivity:

Overexpressed in 94% of PA; rare in adenoid cystic carcinoma

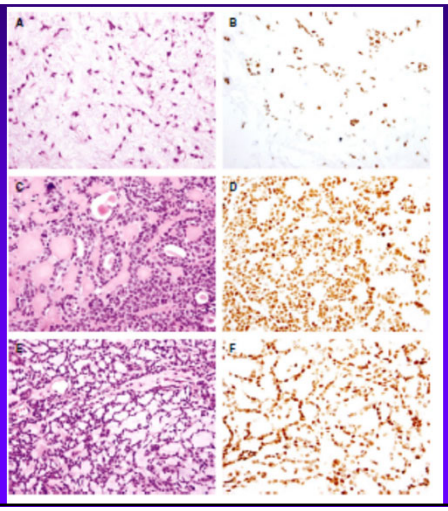
Does not distinguish benign from malignant



Contributed by Dr. J. Krane, BWH

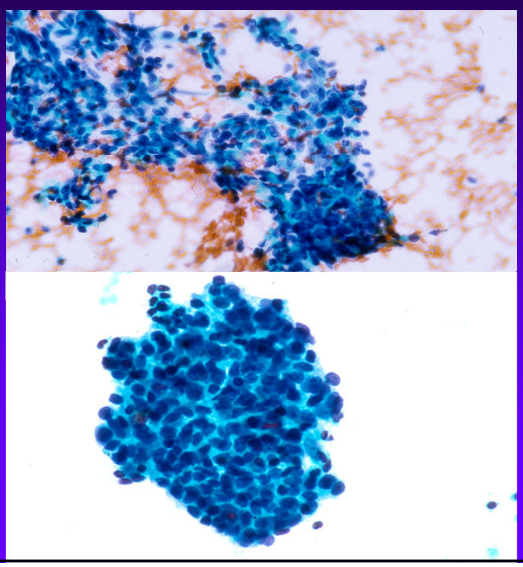
HMGA2 is a specific immunohistochemical marker for pleomorphic adenoma and carcinoma ex-pleomorphic adenoma

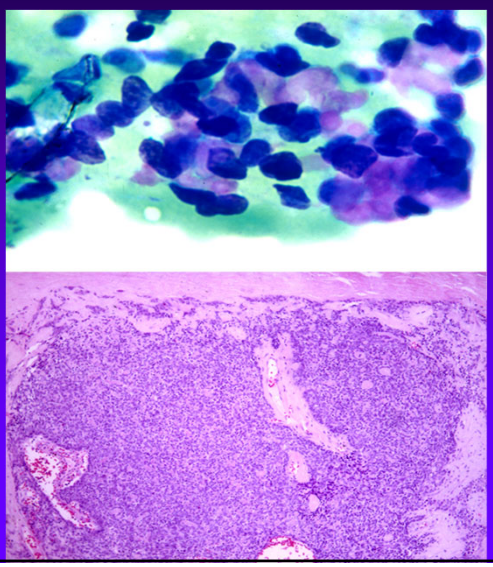
Jeffrey K Mito,[1] Vickie Y Jo,[1] Simion I Chiosea,[2] Paola Dal Cin[1] & Jeffrey F Krane[1]
[1]*Department of Pathology, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, and*
[2]*Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA, USA*



How is basal cell adenoma distinguished from adenoid cystic carcinoma???

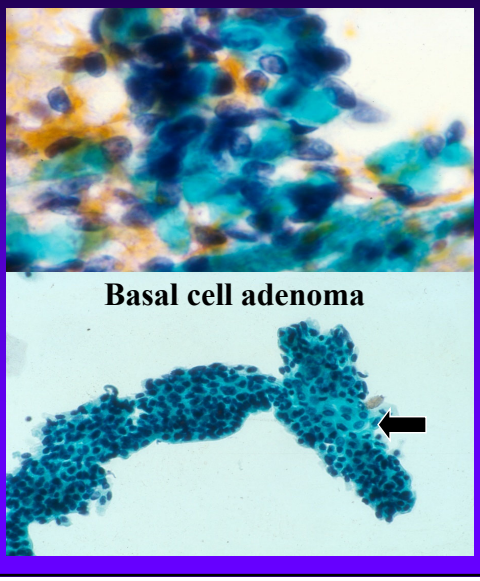
Basal cell adenomas/adenocarcinomas show similarities to adenoid cystic carcinoma





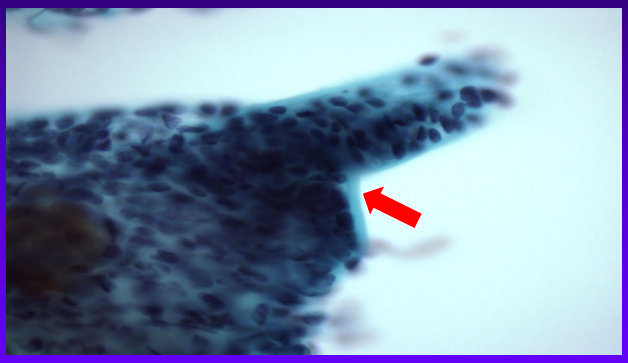
Basal Cell Adenoma/Adenocarcinoma: Distinguishing Features

Matrix droplets, palisading, squamous morules, matrix ribbons favor BCA



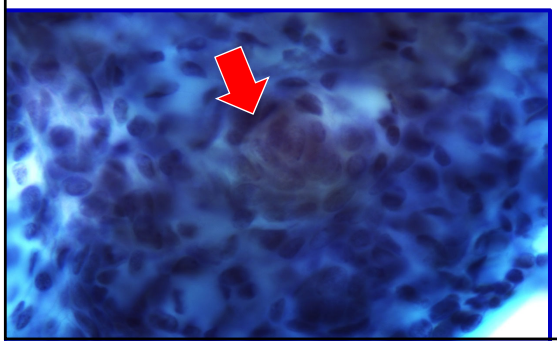
Basal cell adenoma

Solid Pattern

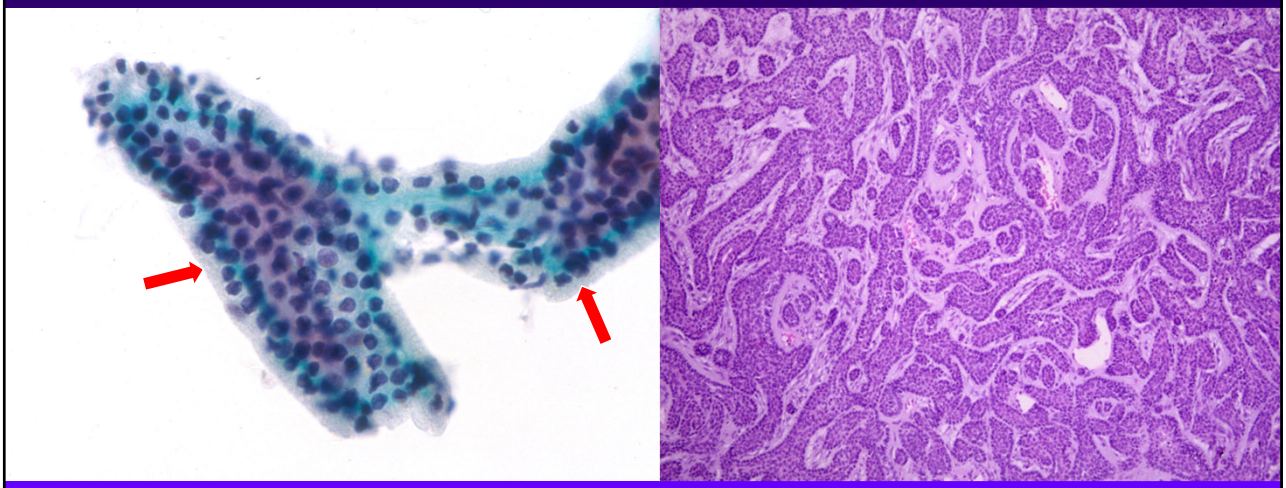


Squamous Morules

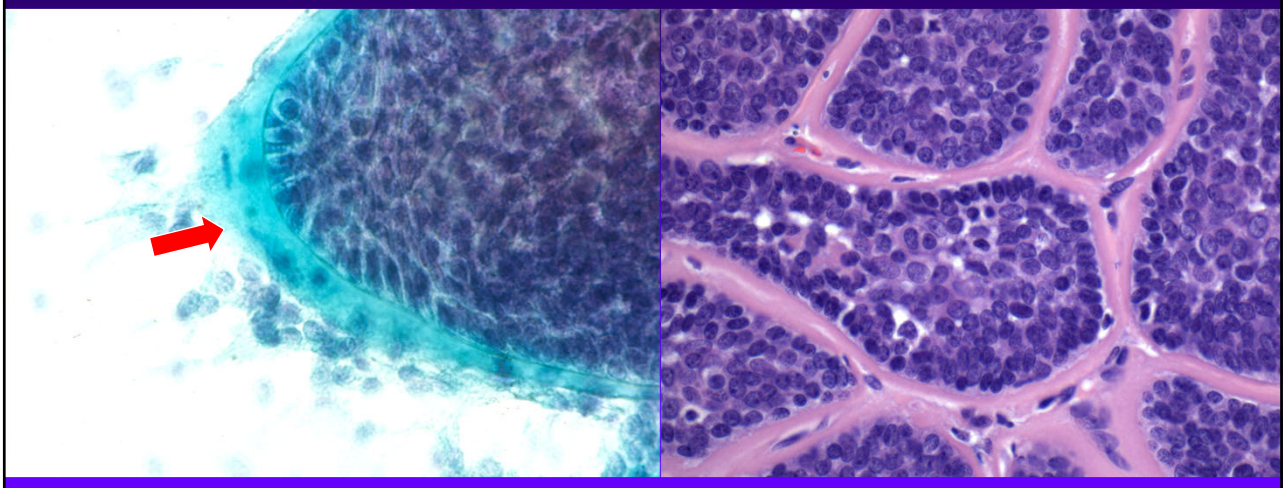
Squamous differentiation is not a feature of adenoid cystic carcinoma!



BASAL CELL ADENOMA & ADENOCARCINOMA:
Tubulotrabecular Type with Peripheral Matrix Ribbon



BASAL CELL ADENOMA & ADENOCARCINOMA:
Membranous Type: Brooke Spiegler Syndrome
Easily distinguished from adenoid cystic carcinoma

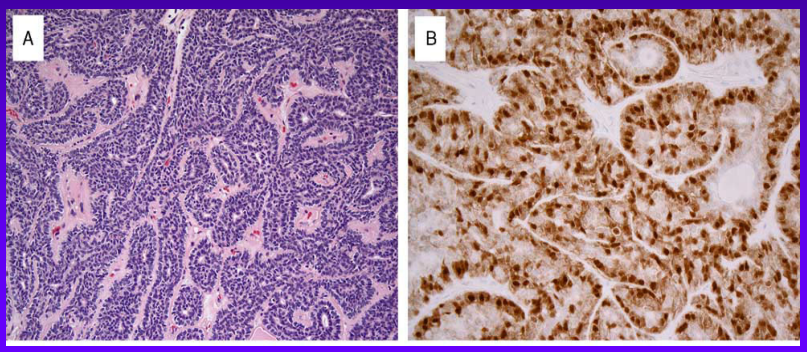


A Useful Ancillary Marker: Nuclear Beta-Catenin in Basal Cell Adenoma

Jo et al. AJSP 2016;40:1143-1150.

Distinctive Patterns of *CTNNB1* (β-Catenin) Alterations in Salivary Gland Basal Cell Adenoma and Basal Cell Adenocarcinoma

Vickie Y. Jo, MD, Lynette M. Sholl, MD,*† and Jeffrey F. Krane, MD, PhD**



Nuclear b-catenin is not a feature of adenoid cystic carcinoma

Molecular Differences Between Basal Cell Adenoma & Adenocarcinoma

> Nat Commun. 2025 May 19;16(1):4657. doi: 10.1038/s41467-025-59871-3.

Wnt/β-catenin activation by mutually exclusive FBXW11 and CTNNB1 hotspot mutations drives salivary basal cell adenoma

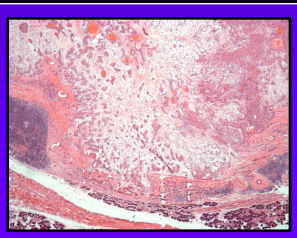
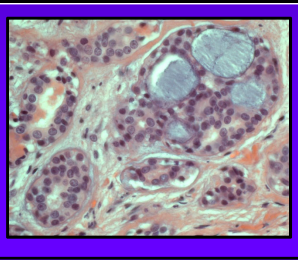
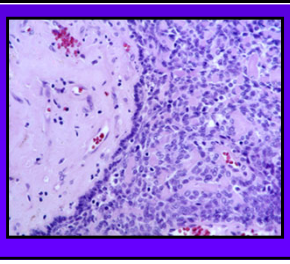
Kim Wong [1], Justin A Bishop [# 2], Ilan Weinreb [# 3 4], Marialetizia Motta [# 5], Martin Del Castillo Velasco-Herrera [1], Emanuele Bellacchio [5], Ingrid Ferreira [1],

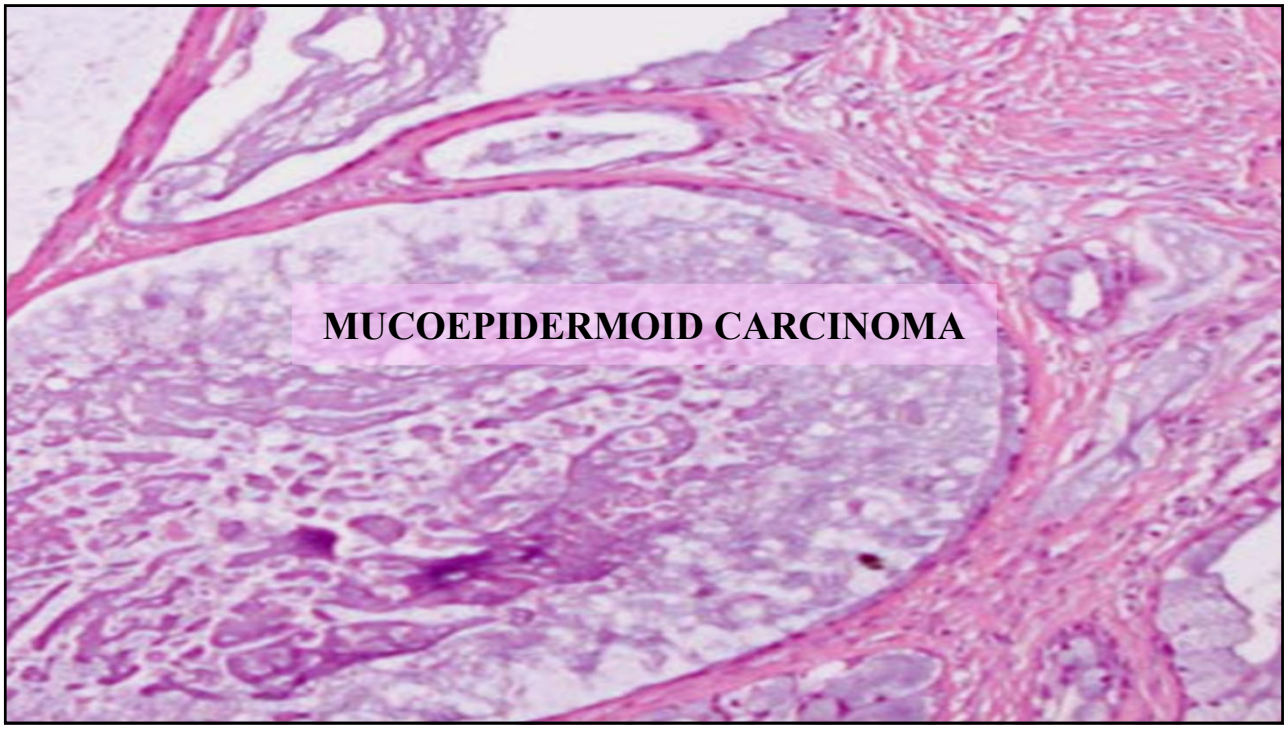
2025

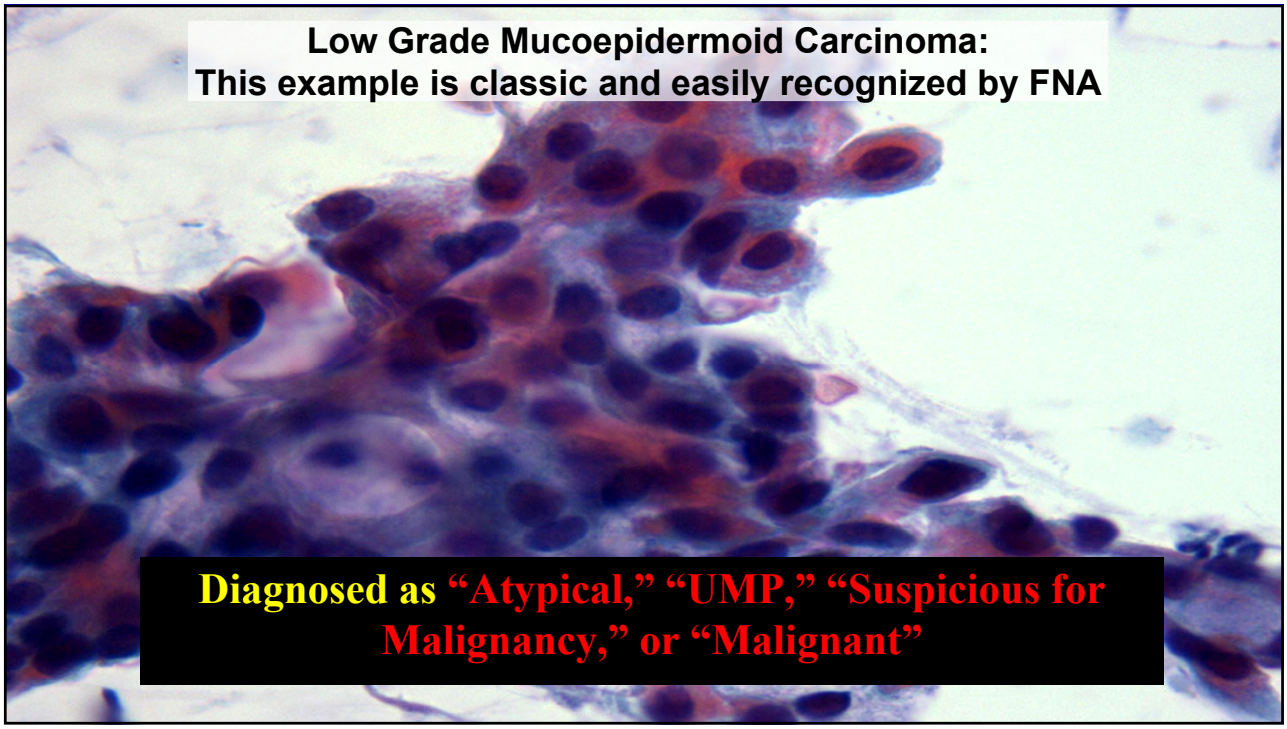
- **BCA**
  - **Recurrent FBXW11 missense mutation (p.F517S)**
  - **CTNNB1 p.I35T gain-offunction (GoF) mutation**
- **BCAC**
  - **Hotspot DICER1 and HRAS mutations**
  - **Putative driver mutations affecting PI3K/AKT and NF-κB signalling pathway genes**

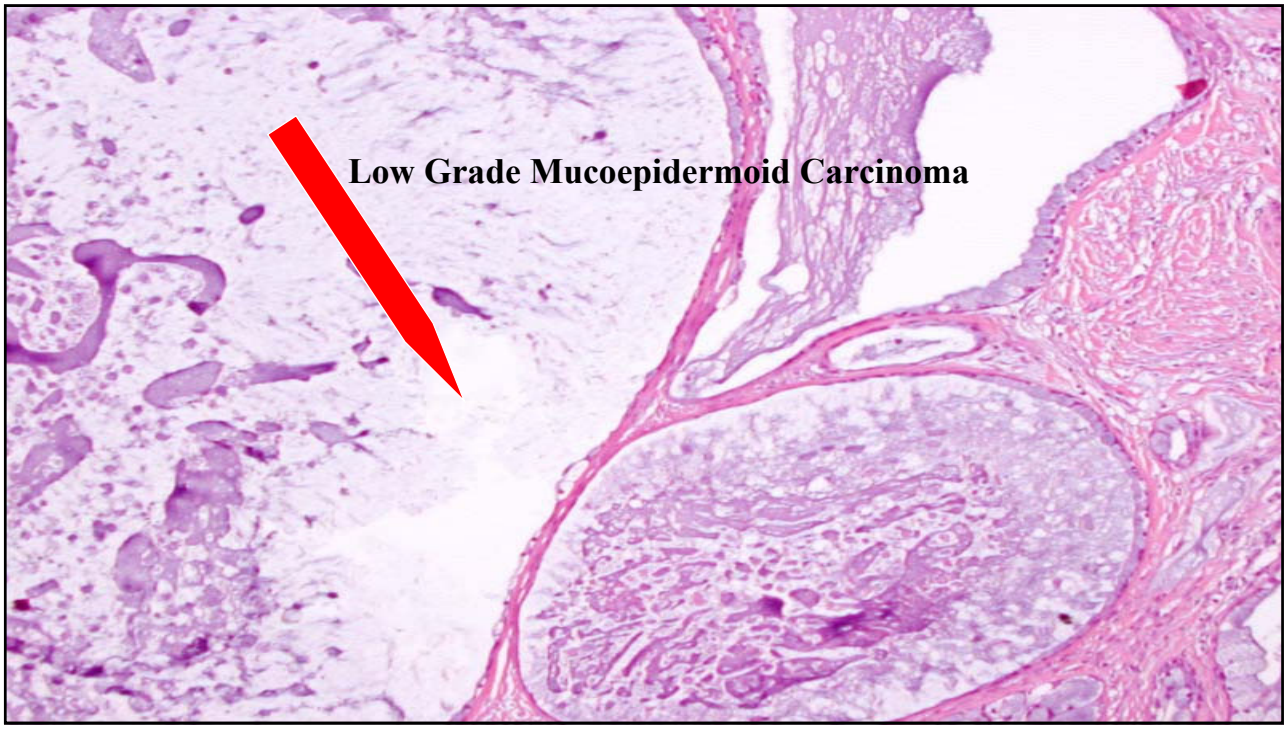
Summary: A Challenging DDX
PA vs AdCC vs BCA

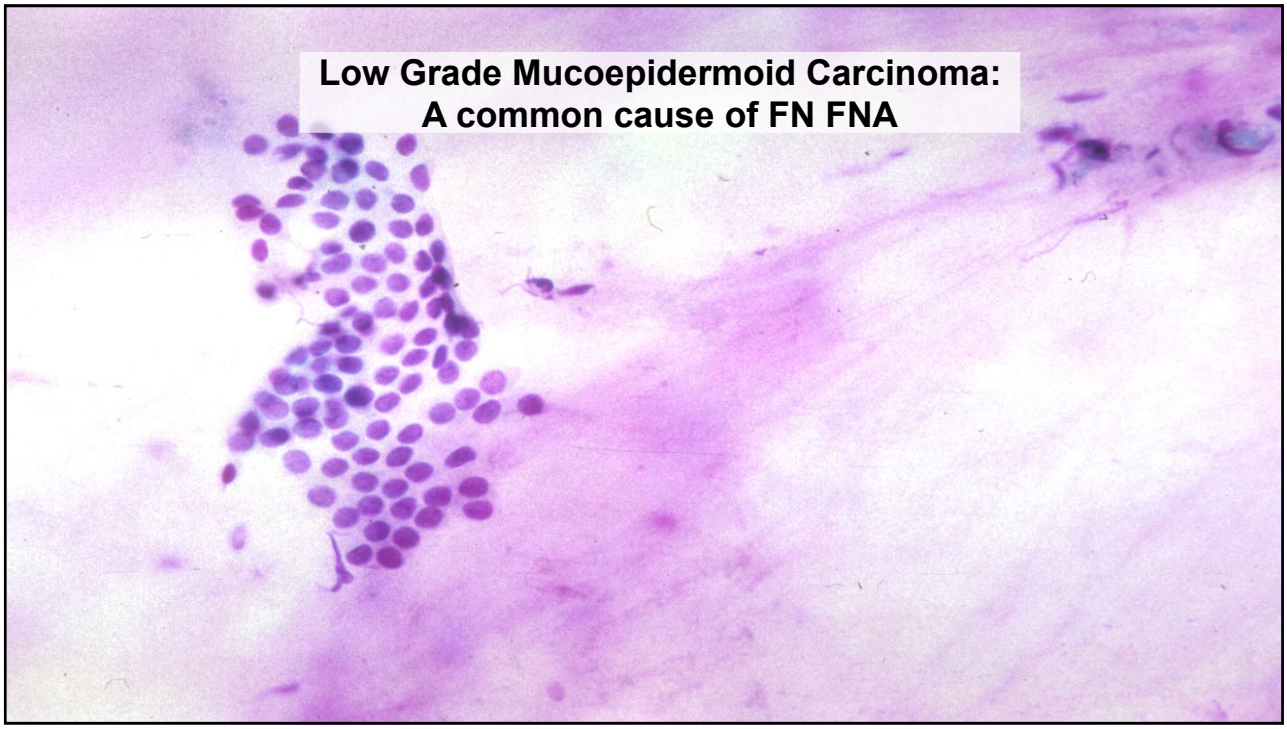
PA	AdCC	BCA
• Fibrillar matrix; embedded cells	• Matrix spheres sharp; acellular	• Peripheral matrix ribbons; droplets
• PLAG1+/HMGA2+	• MYB+/CD117+	• Nuclear b-Catenin+
• Myoep-predominant	• Small basaloid cells	• Palisading; squamous morules




Low Grade Mucoepidermoid Carcinoma:
This example is classic and easily recognized by FNA
Diagnosed as "Atypical," "UMP," "Suspicious for Malignancy," or "Malignant"


Low Grade Mucoepidermoid Carcinoma



Mucoepidermoid Carcinoma Immunoprofile

Non-Specific Immunohistochemistry:

- **Positive for:**
  - **Keratin 5,6,7,8,19**
  - **EMA**
  - **CEA**
  - ***p63**
- **Negative for:**
  - **SM actin**
  - **Calponin**
  - **S-100**
  - **SOX-10**

Mucoepidermoid Carcinoma:

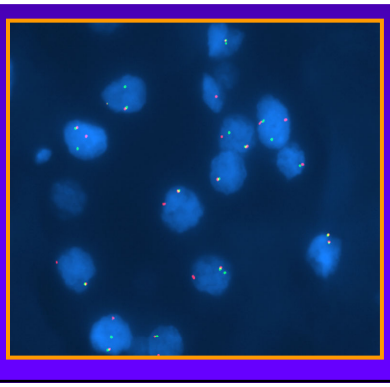
Among the most useful SG molecular probes for FNA and small biopsies

A Reappraisal of the MECT1/MAML2 Translocation in Salivary Mucoepidermoid Carcinomas

Raja R. Seethala, MD, Sanja Dacic, MD, PhD, Kathleen Cieply, MS, Lindsey M. Kelly, BS, and Marina N. Nikiforova, MD

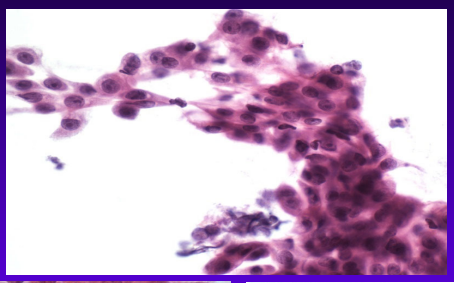
Cytogenetics:

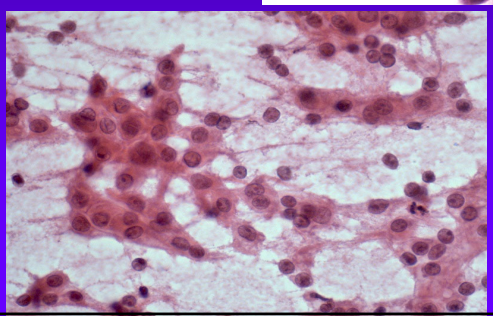
- t(11:19) translocation
- MECT1/MAML2
- FISH or NGS
- More common in low grade
- Often a better prognosis
  - >75%
  - LG-IG 75%, HG 32%

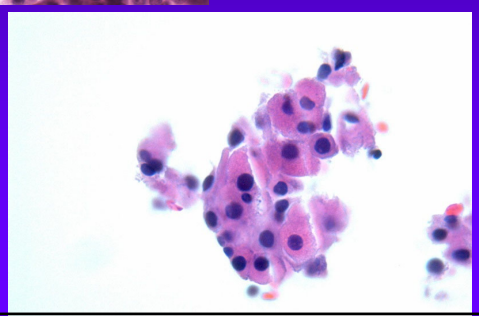


Mucoepidermoid Carcinoma:

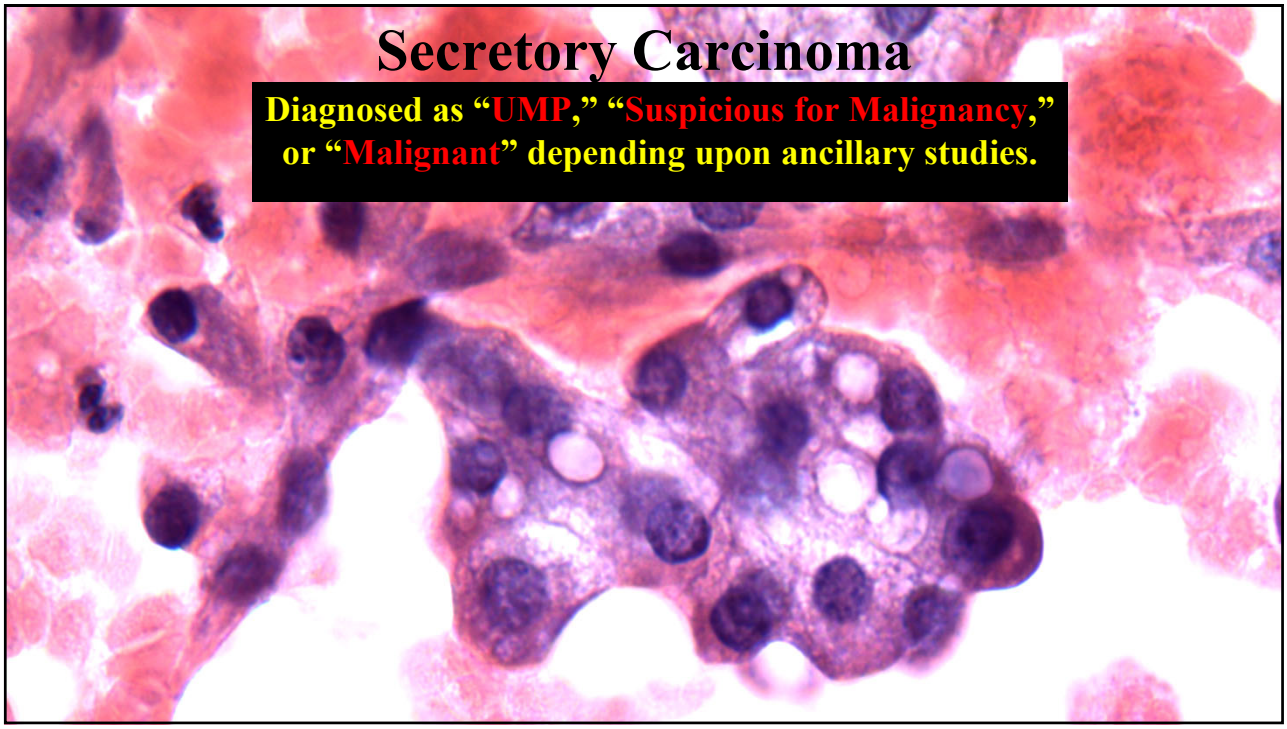
3 FNA examples positive for MAML2 fusion





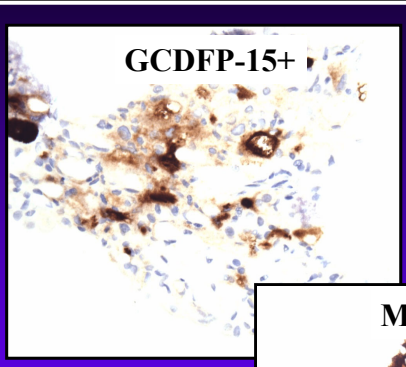


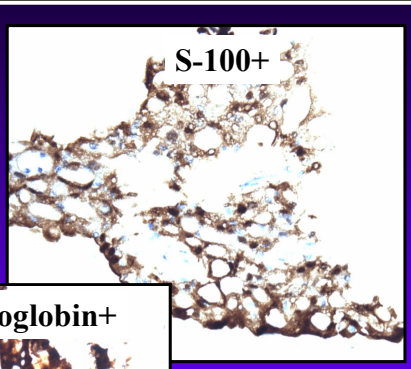

Secretory Carcinoma

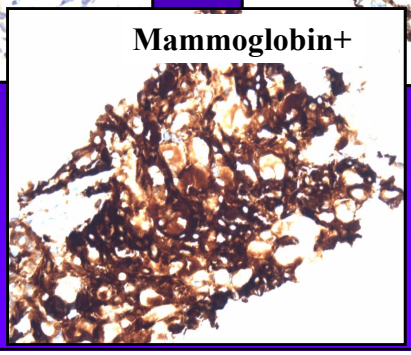

Secretory Carcinoma
Diagnosed as "UMP," "Suspicious for Malignancy," or "Malignant" depending upon ancillary studies.

Secretory Carcinoma: Immunohistochemical Studies

GATA-3+, S-100+, Mammaglobin+, GCDFP-15+, Pan-TRK+



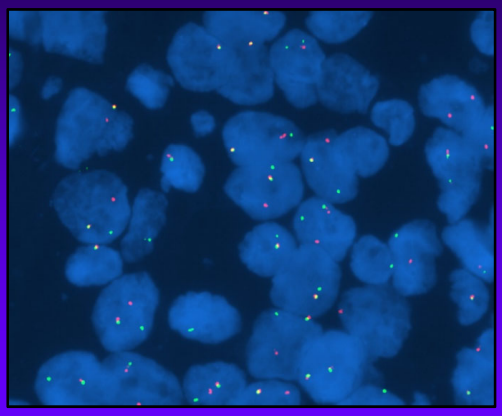




FNA of Secretory Carcinoma and NTRK: Detection of Fusion is Diagnostic

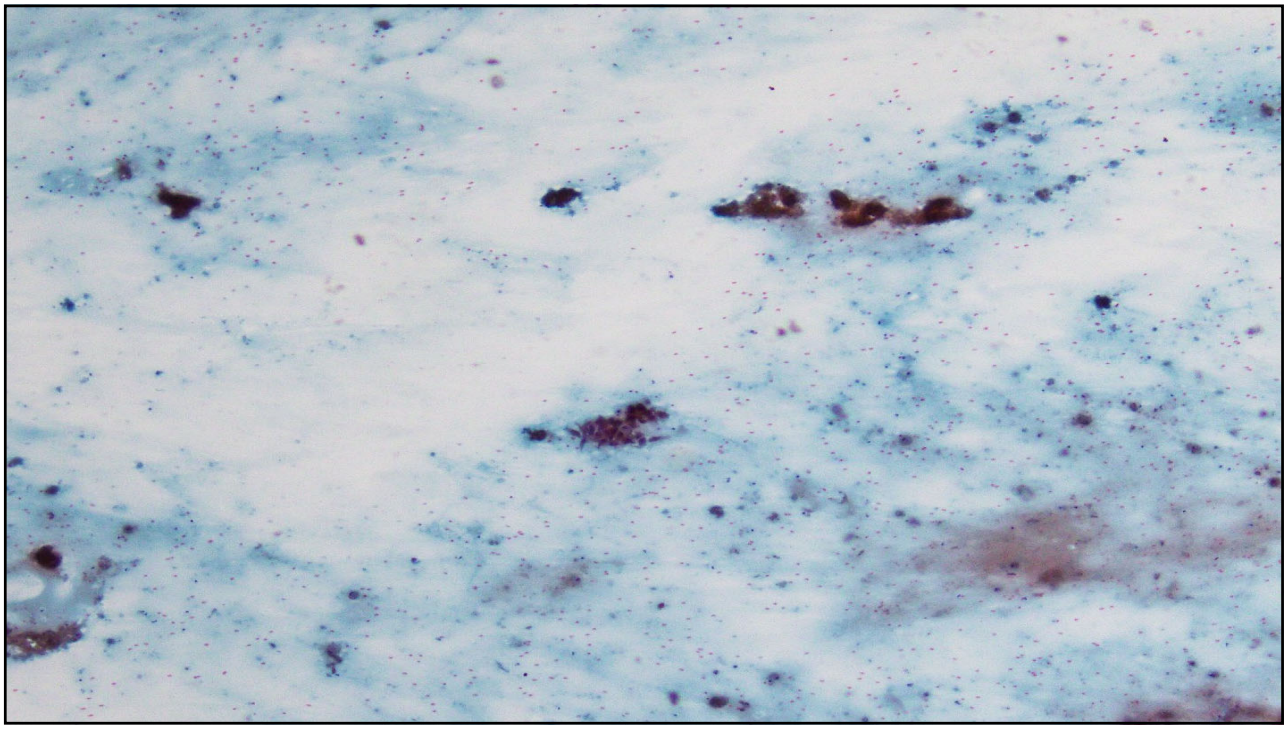
- ETV6-NTRK3 Fusion T(12:15)(p13;q25)
- Pan-TRK antibody available
- FDA-approved TRK inhibitors
  - Larotrectinib
  - Entrectinib (side effects)
  - 56-76% response rate

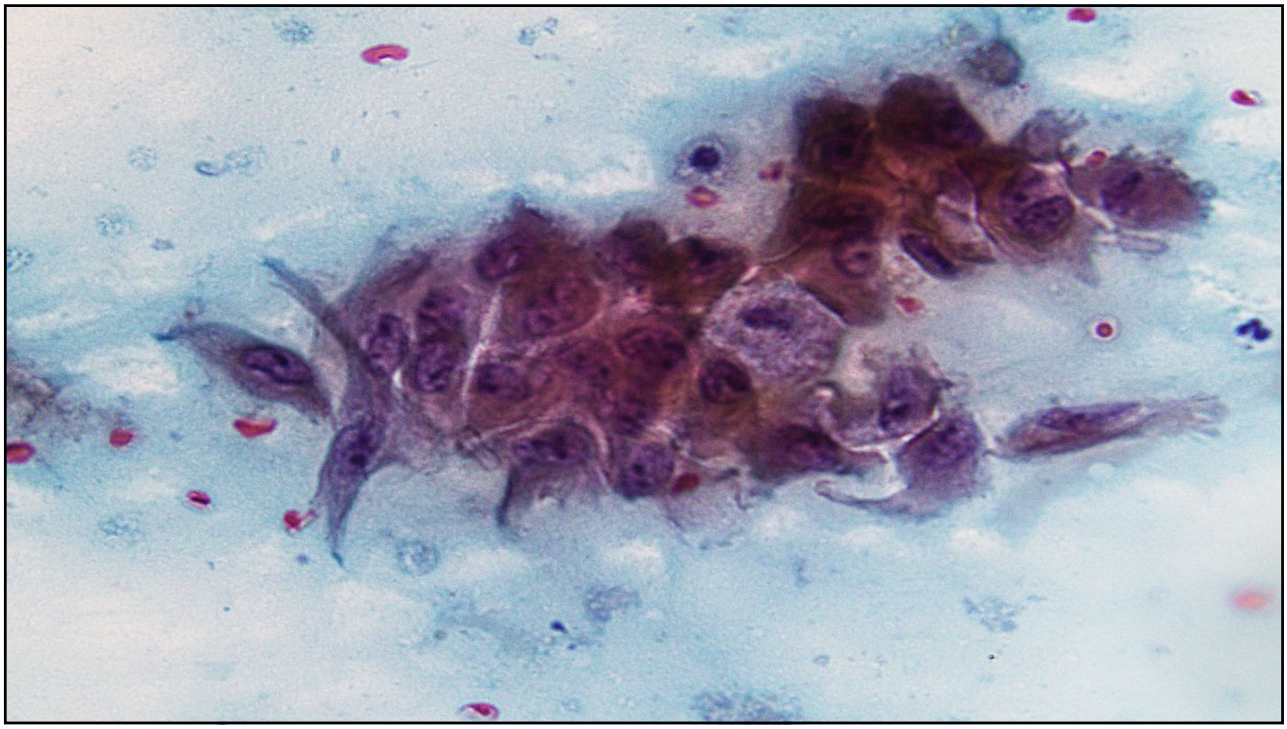
FISH analysis for ETV6

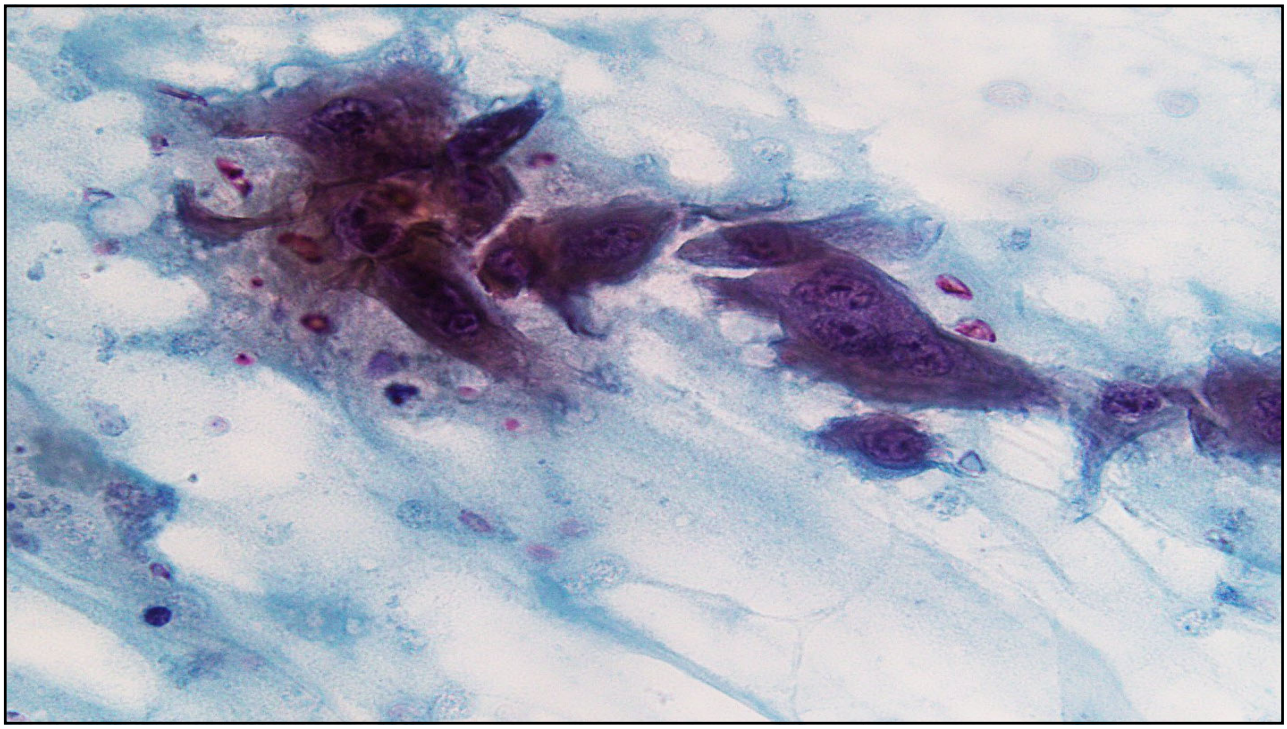


One last pitfall to avoid...

A 75 year-old man with bilateral parotid nodules including an enlarging 3.0 cm parotid mass. An FNA is performed.







What is your FNA Diagnosis?

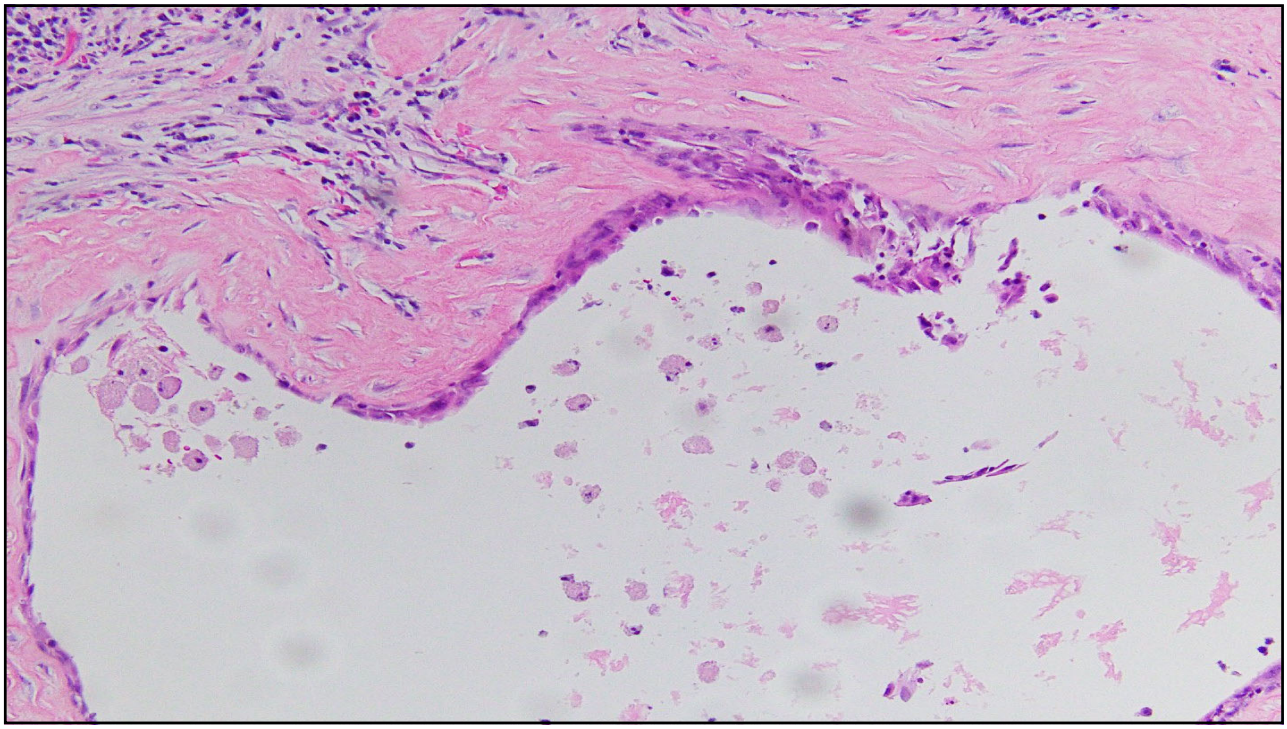
Cytologic Diagnosis:

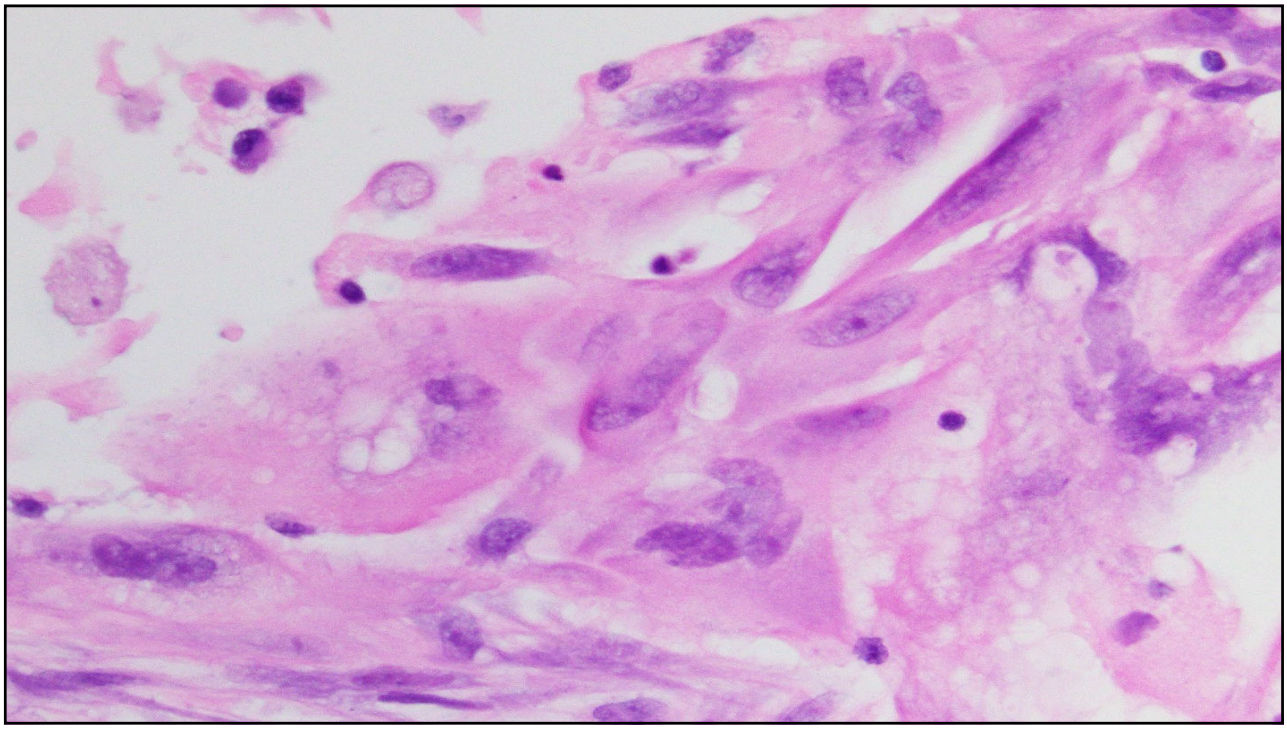
SUSPICIOUS FOR MALIGNANCY

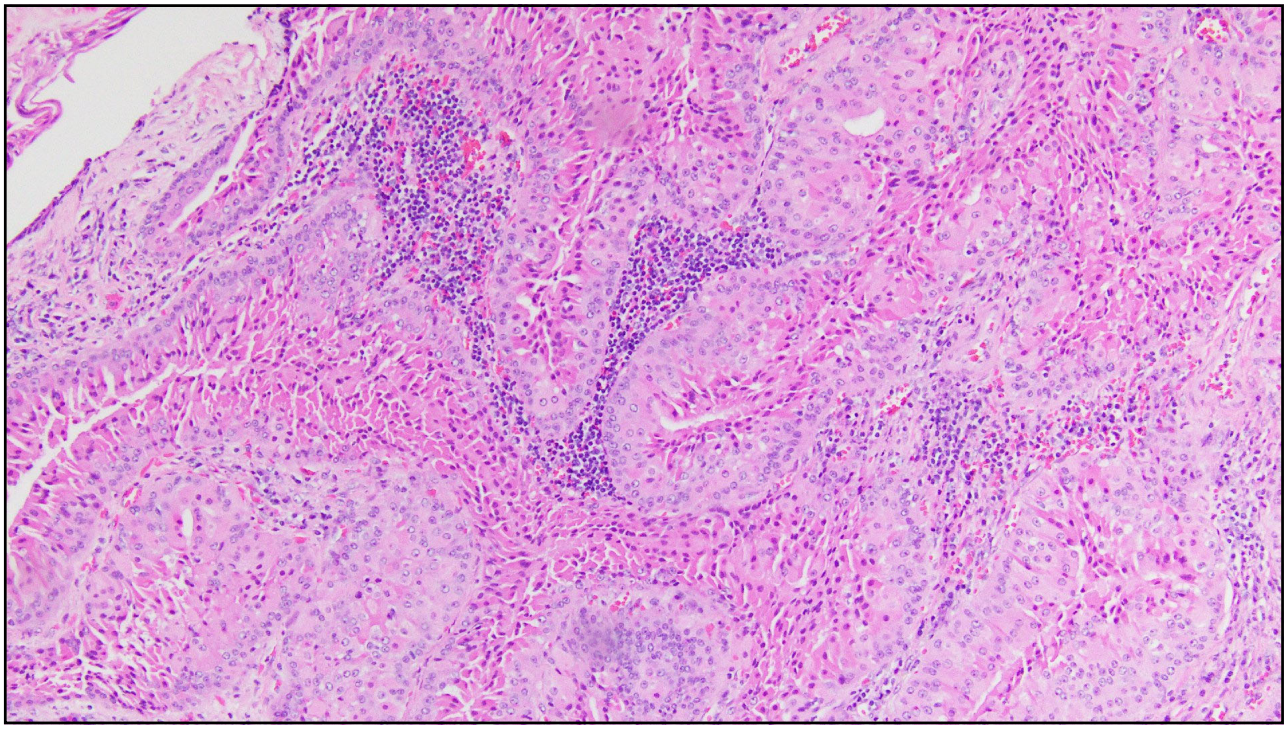
Atypical spindled cell neoplasm suspicious for malignancy.

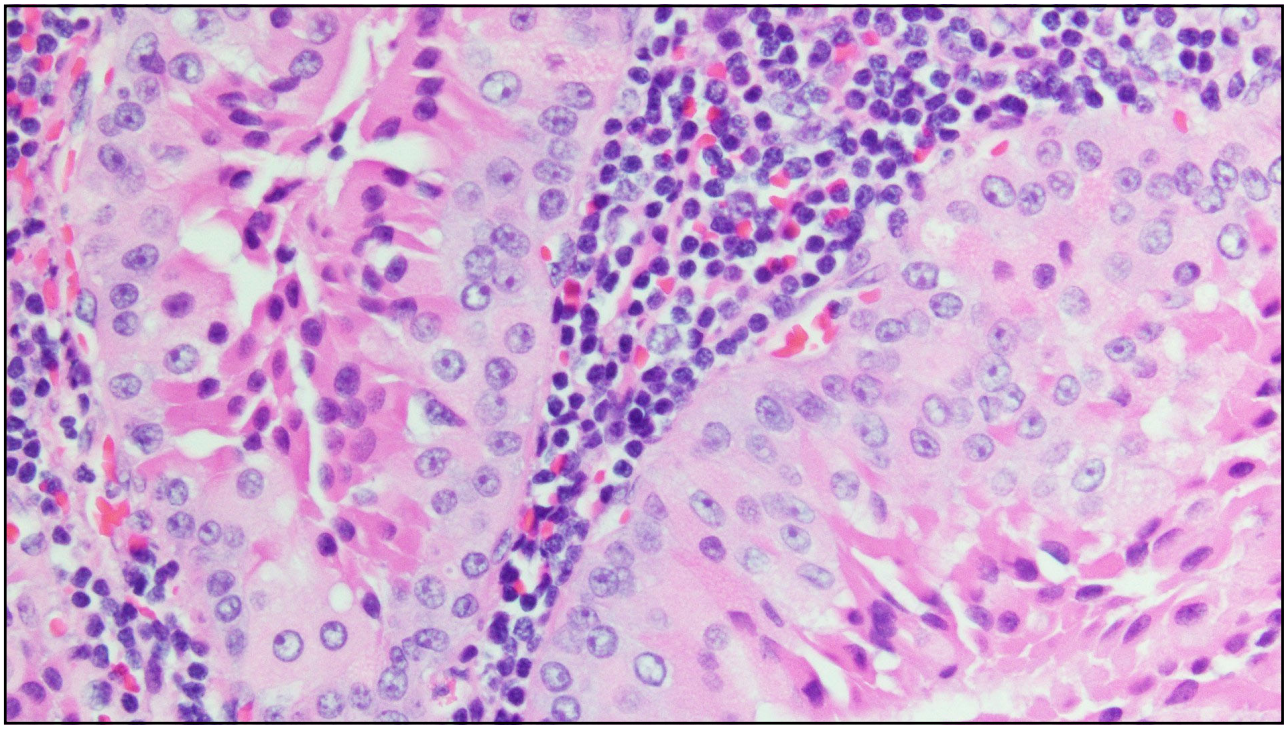
IHC was non-specific (ker 7+, p63+). Molecular profiling was negative for mutations and fusions.

The tumor was surgically excised by superficial parotidectomy.









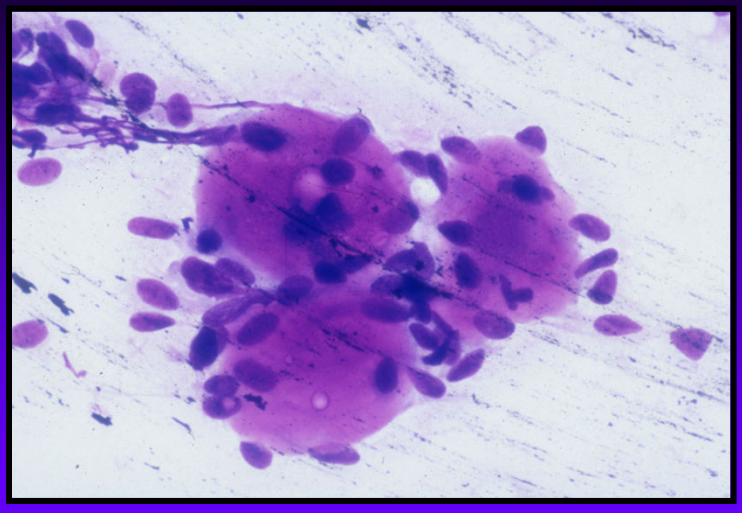
Histologic Diagnosis:

Warthin Tumor, infarcted with metaplastic features.

Beware of Warthin Tumor with metaplastic changes.

Summary

- The Milan System and the upcoming WHO System for HN offer reliable reporting formats for salivary gland
- Basaloid tumors such as AdCC are problematic for FNA and have important implications for treatment and prognosis.
- Careful attention to cytologic features can strongly suggest the diagnosis.
- Selective ancillary studies (IHC and/or molecular) can be applied to SG FNA specimens to improve accuracy.



THANK YOU!