New Classifications for Cytology: The Milan System for Salivary Gland (One Year In)

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Objectives

- Review the bases for the Milan classification system
- Identify the morphological criteria and pitfalls for various benign and neoplastic entities
- Demonstrate the role of ancillary immunocytochemical and molecular tests in lesions of these sites
- Recognize the management options of lesions in these sites

The Benefits of a Uniform Reporting System for Salivary Gland Cytopathology

- Improve communication
  - Between pathologists, clinicians and patients
- Improve patient care
  - Help standardize ROM and clinical management
- Facilitate cytologic-histologic correlation
- Facilitate research and sharing of data from different laboratories for collaborative studies

The Milan System for Reporting Salivary Gland Cytopathology

Assembled in 2015

Sponsored by the ASC and the IAC

Goal to produce a practical, user-friendly and internationally accepted classification system

Evidence-based

Anticipated that the classification system and ROM for the diagnostic categories will be further refined as more data is available in the literature

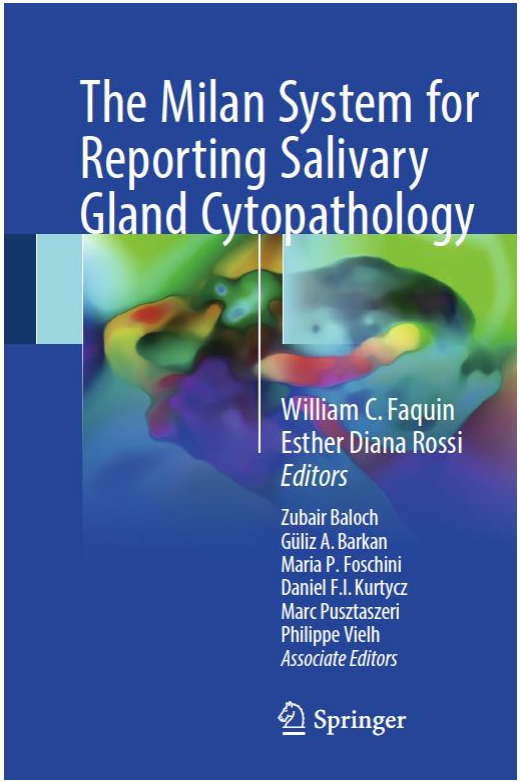
The Milan System for Reporting Salivary Gland Cytopathology

Milan Atlas

45 Members from 15 countries

Cytopathologists, Surgical Pathologists, Molecular Pathologists, ENT Surgeons

Published 2/2018



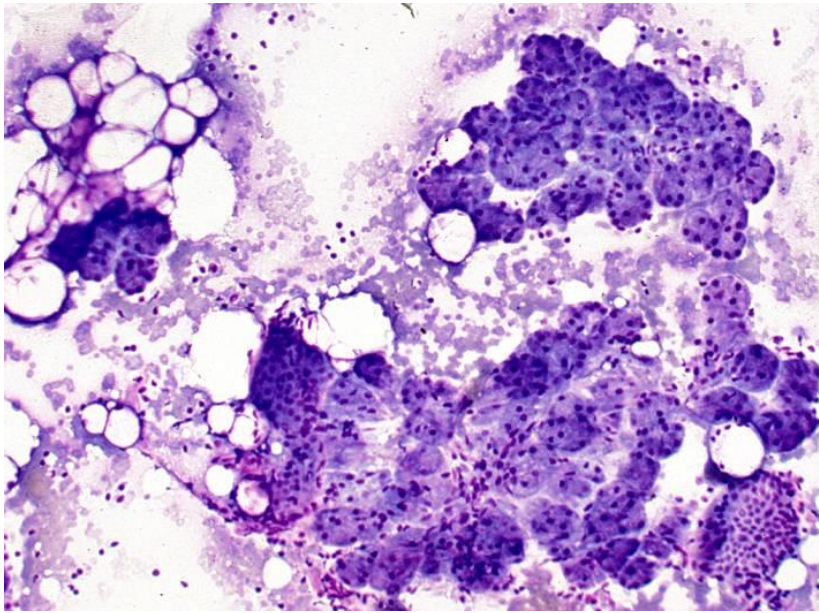
The Milan System for Reporting Salivary Gland Cytopathology

Classification Scheme

1) Non-Diagnostic
2) Non-Neoplastic
3) Atypia of Undetermined Significance (AUS)
4) Neoplasm:
    a) Benign
    b) Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)
5) Suspicious for Malignancy
6) Malignant

Non-Diagnostic

- "…for qualitative and/or quantitative reasons provides insufficient diagnostic material to provide an informative interpretation"
- Poorly preserved slides with artifacts that preclude evaluation
- Includes aspirates with benign elements only, in the setting of a defined mass
- Includes non-mucinous cyst contents without epithelial elements



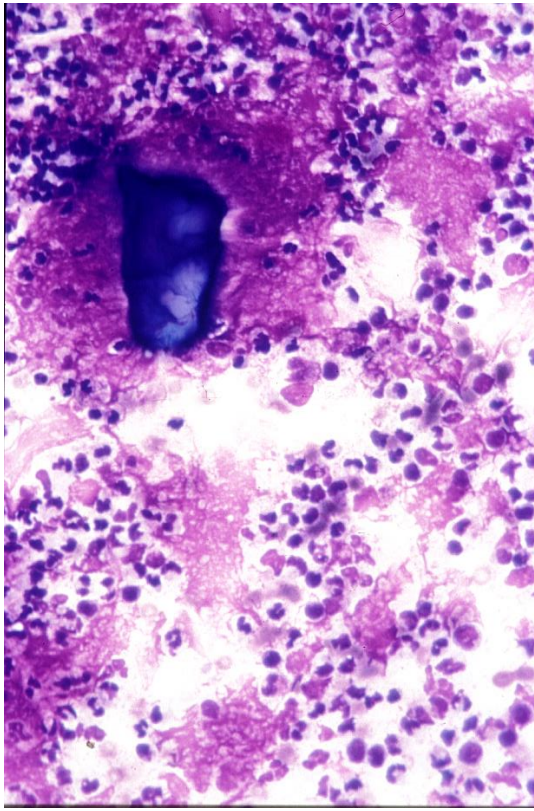
Non-Diagnostic

- Adequacy criteria not clearly established
  - “recommended that until more data is available a minimum of 60 lesional cells, could be used as a reasonable and objective measure of adequacy”
- Excludes cases with significant cytologic atypia
  - AUS
- Excludes mucinous cysts without epithelium
  - AUS
- Excludes abundant inflammatory cells
  - Non-neoplastic
- Excludes aspirates with matrix elements
  - ?Neoplasm:Benign or AUS

Non-Neoplastic

<u>Specimens lacking evidence of a neoplastic process:</u>

- Inflammatory
- Metaplastic
- Reactive
- Examples:
  - Acute, chronic, and granulomatous sialadenitis
  - Sialadenosis
  - Reactive lymph nodes (flow cytometry desirable)
- Clinico-radiological correlation is essential to ensure that the specimen is representative of the lesion



Atypia of Undetermined Significance

Cannot entirely exclude a neoplasm

Heterogeneous category

A majority will be reactive atypia or poorly sampled neoplasms

Specimens are often compromised

- (eg, air-drying, blood clot)

Should be used rarely

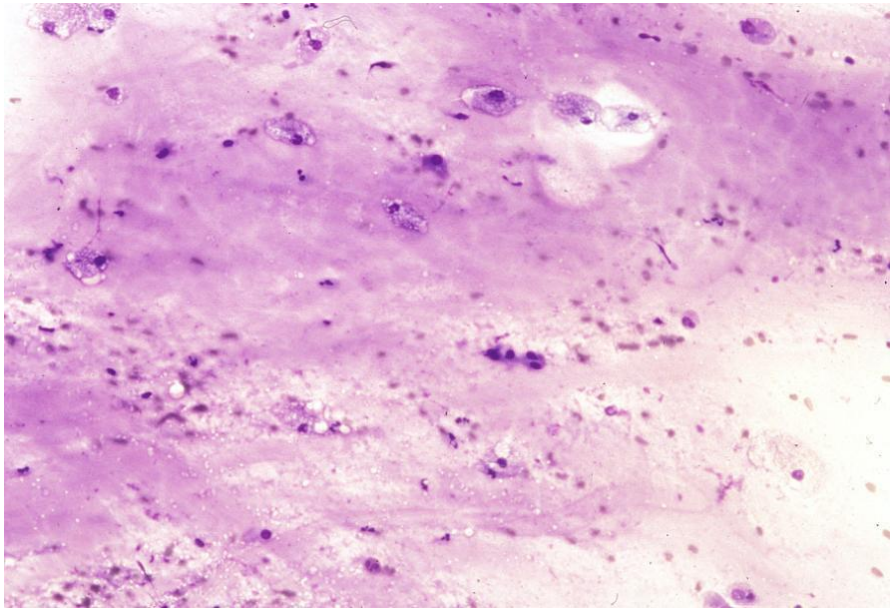
- (<10% of all salivary gland FNAs)

Atypia of Undetermined Significance (AUS)

Cannot entirely exclude a neoplasm

- Reactive/reparative atypia
- Metaplastic changes
- Low cellularity
- Preparation artifact
- Lymphoid lesion

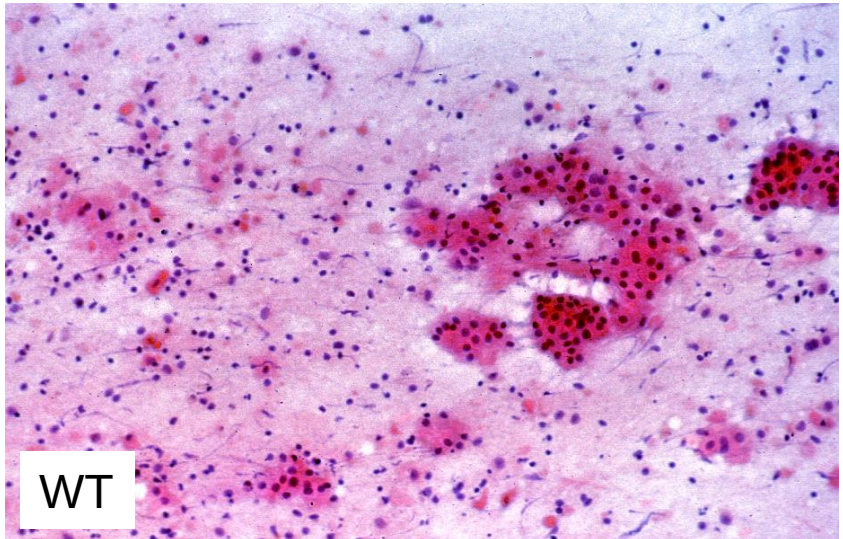
Mucinous cysts with no or limited cellularity

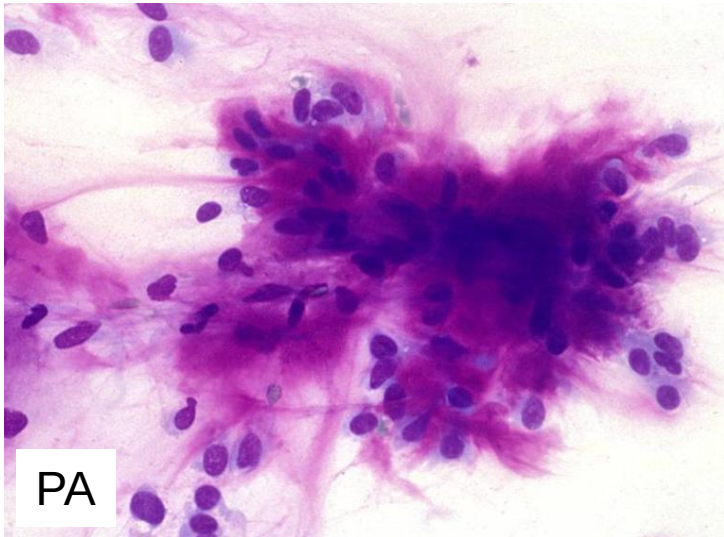


Neoplasm

A) Neoplasm: Benign

- Reserved for clear-cut benign neoplasms
- Includes classic cases of PA, WT, lipoma, etc…

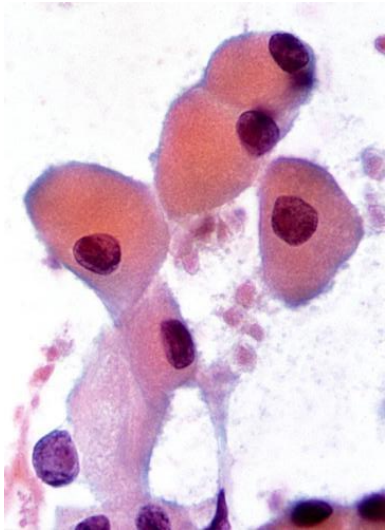


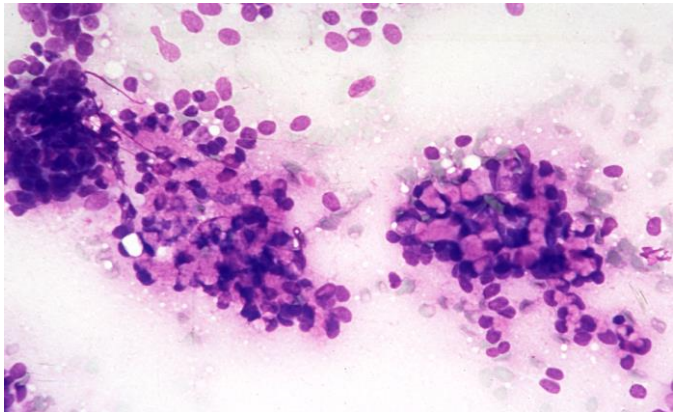


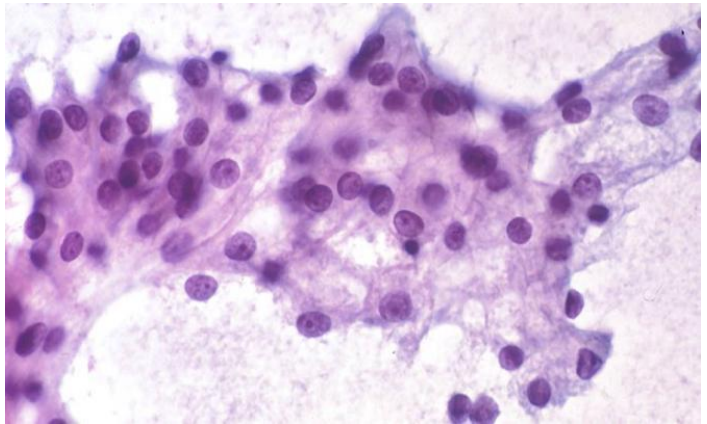
Neoplasm

B) Neoplasm: 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
- Three major differentials:
  - -Basaloid
  - -Oncocytic
  - -Clear cell







Suspicious for Malignancy

Aspirates which are highly suggestive of malignancy but not definitive

Often high-grade carcinomas with limited sampling or other limitation

Or neoplastic with limited cytologic features suspicious for a specific malignancy, but not diagnostic (e.g., adenoid cystic, mucoepidermoid, acinic)

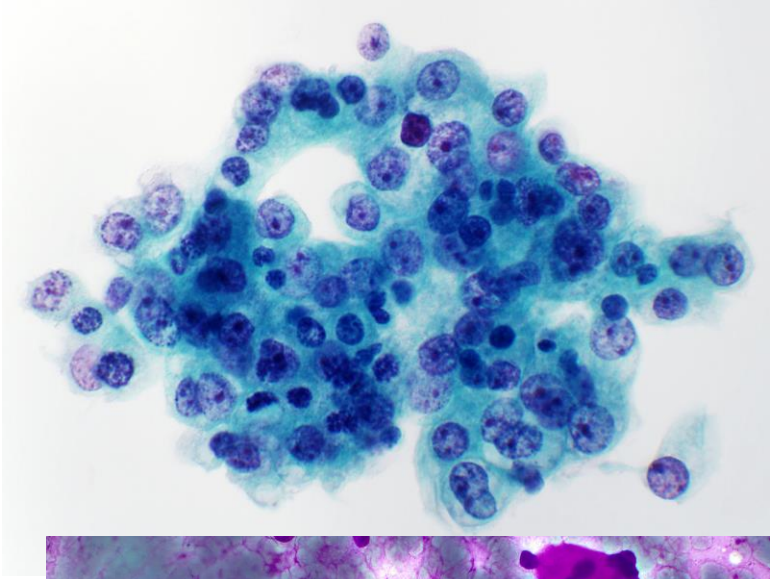
Malignant

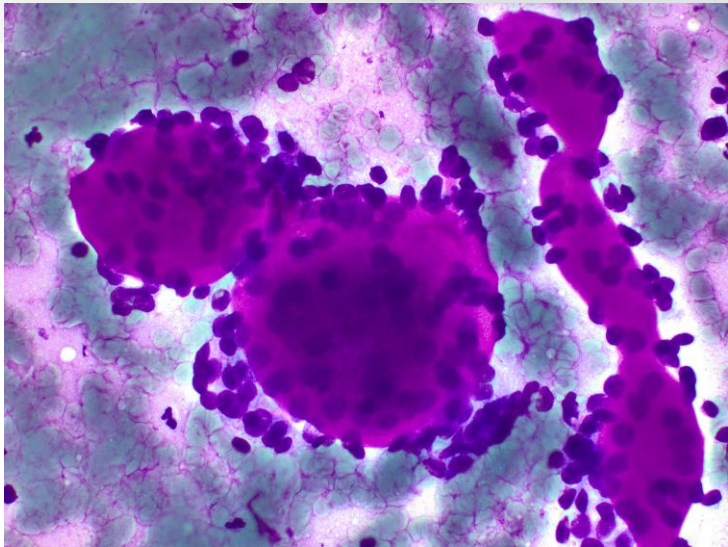
Aspirates which are diagnostic of malignancy

Sub-classify into specific types and grades of carcinoma: e.g. low grade vs high grade

"Other" malignancies

- Lymphomas
- Sarcomas
- Metastases





The Milan System for Reporting Salivary Gland Cytopathology

Overview of Terminology and Reporting

Diagnostic Category	ROM	Management
I. Non-Diagnostic	25%	Clinical and radiologic correlation/ repeat FNA
II. Non-Neoplastic	10%	Clinical follow-up and radiologic correlation
III. Atypia of Undetermined Significance (AUS)	20%	Repeat FNA or surgery
IV. Neoplasm		
A. Benign	<5%	Surgery or Clinical F/U
B. Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)	35%	Surgery
V. Suspicious for Malignancy	60%	Surgery
VI. Malignant	90%	Surgery

Ancillary testing

What are the tests?

Which entities are they helpful in diagnosing?

When should we use them?

Surgical Management of Parotid Tumors

- Facial nerve is key
- Benign tumors and low-grade carcinomas
  - “Lumpectomy”/Superficial parotidectomy
  - Observation an option in subset (e.g., WT in elderly patient)
- High-grade carcinomas
  - Total parotidectomy
  - Often accompanied by neck dissection
  - Typically with radiation therapy

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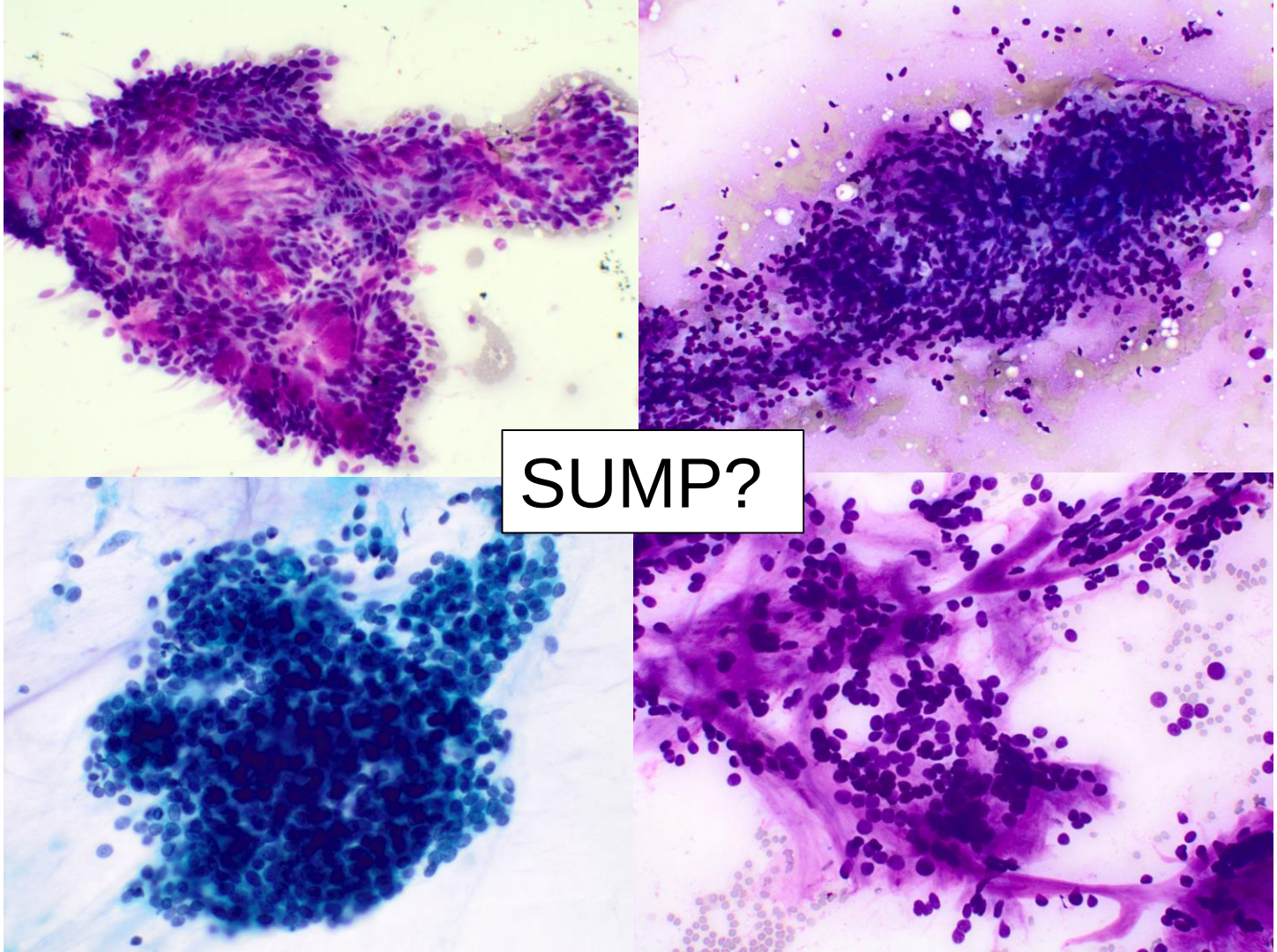

SUMP?

Ancillary tests

Histochemical stains

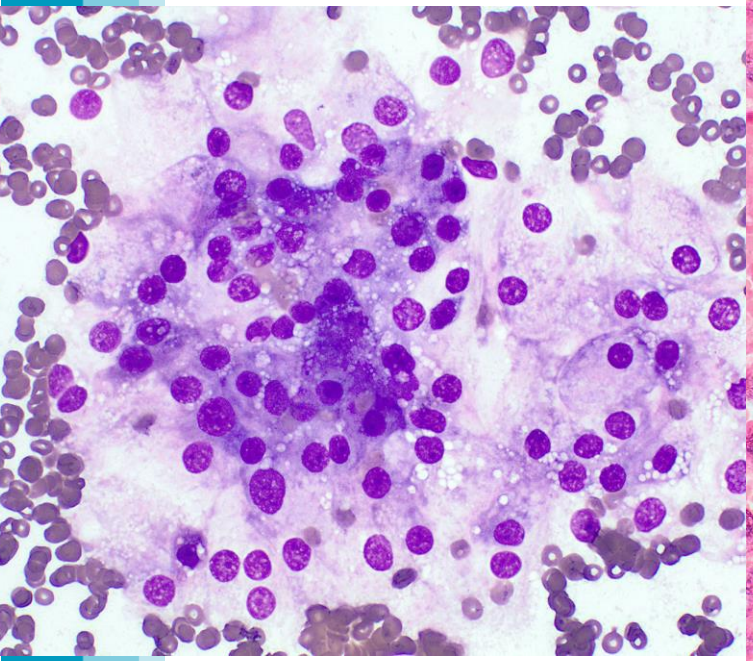
Immunochemical stains

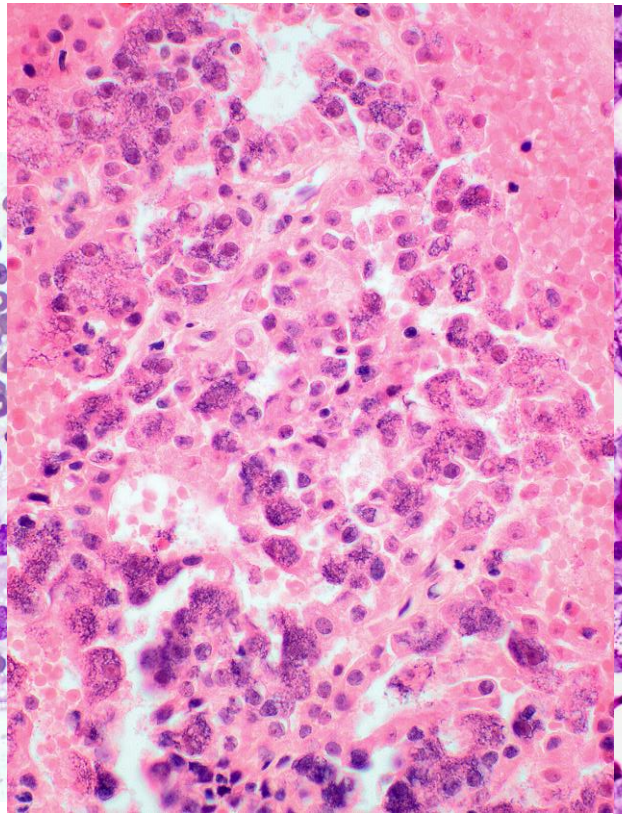
FISH, Cytogenetics, NGS

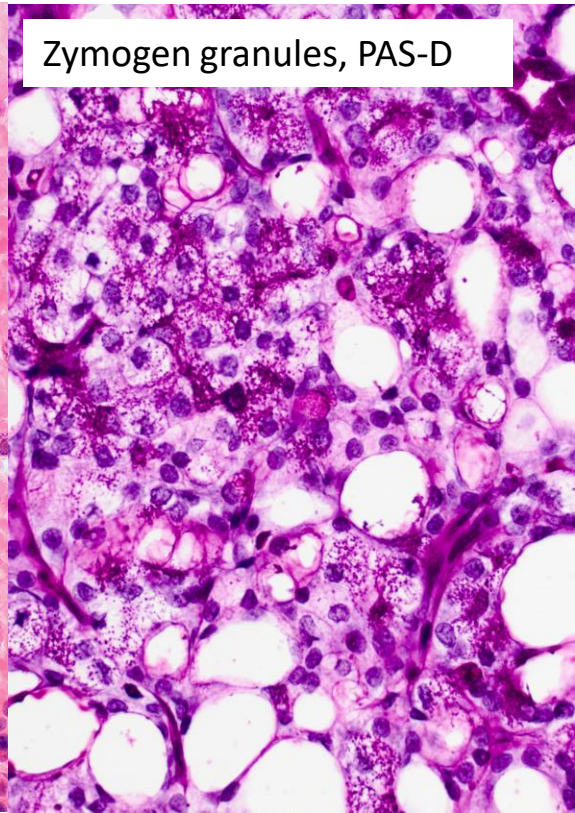
(Flow cytometry)

Histochemical stains

Acinic cell carcinoma





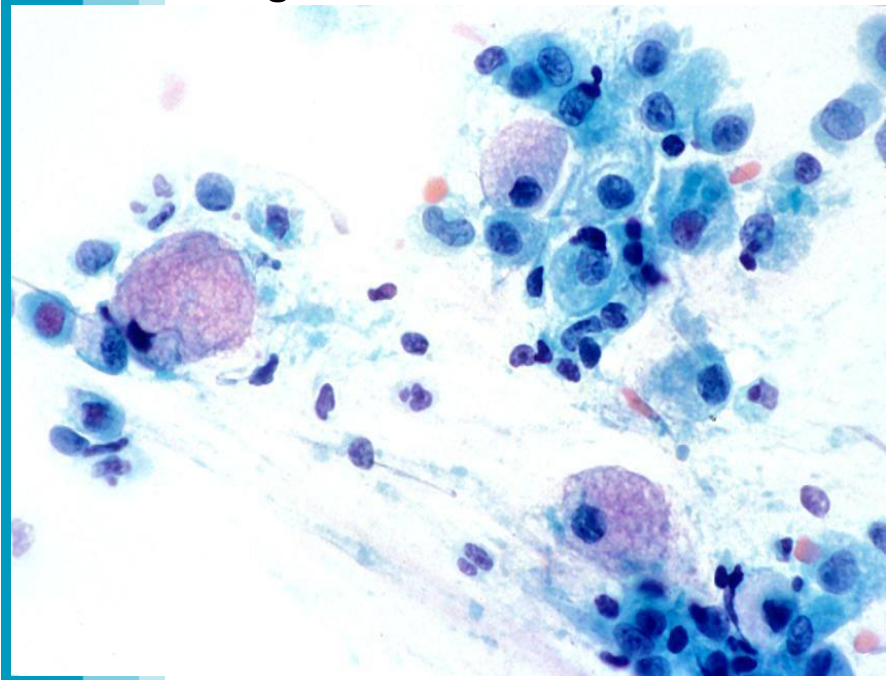


Zymogen granules, PAS-D

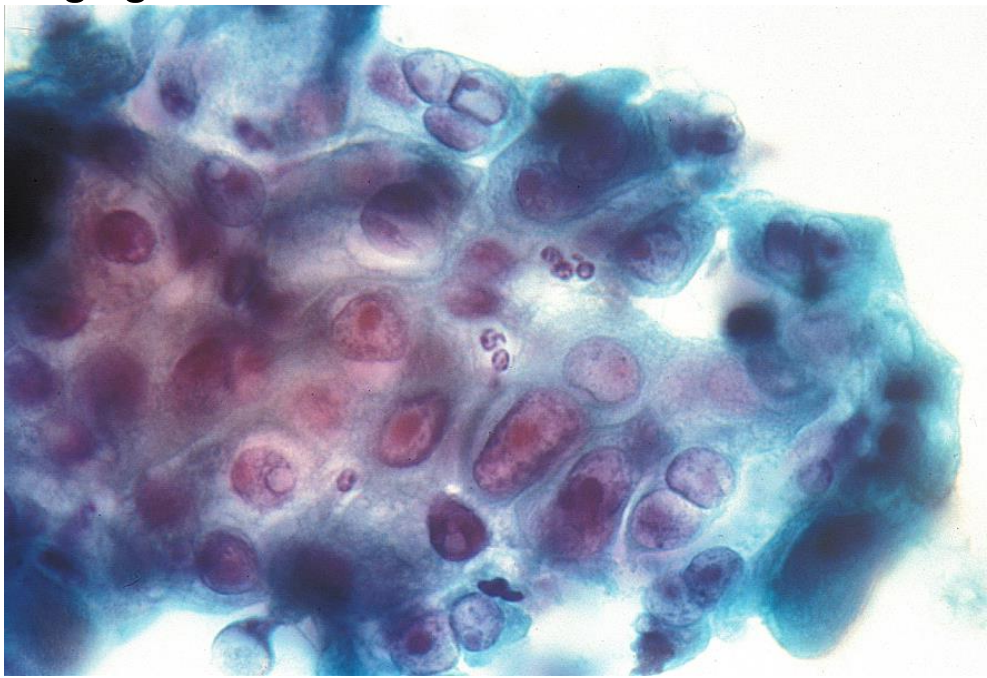
Histochemical stains

Mucoepidermoid carcinoma - Mucicarmine

Low-grade



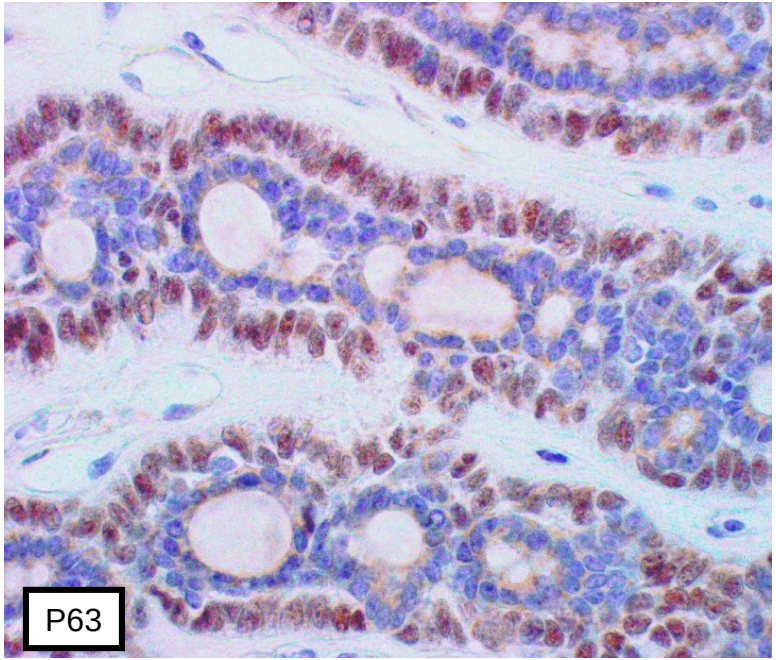
High-grade

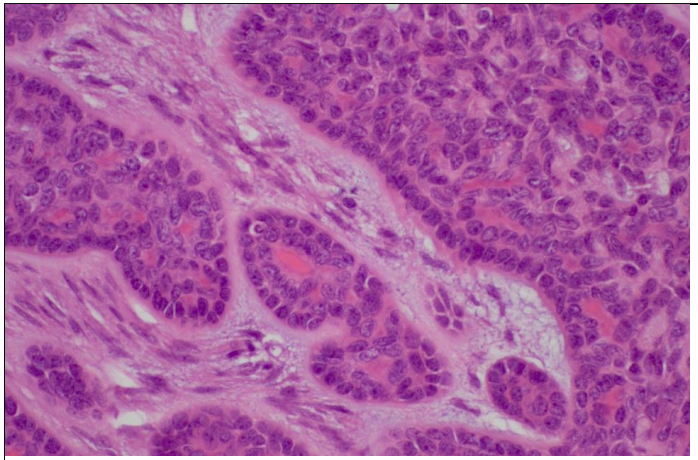


Immunochemistry: Myoepithelial cells

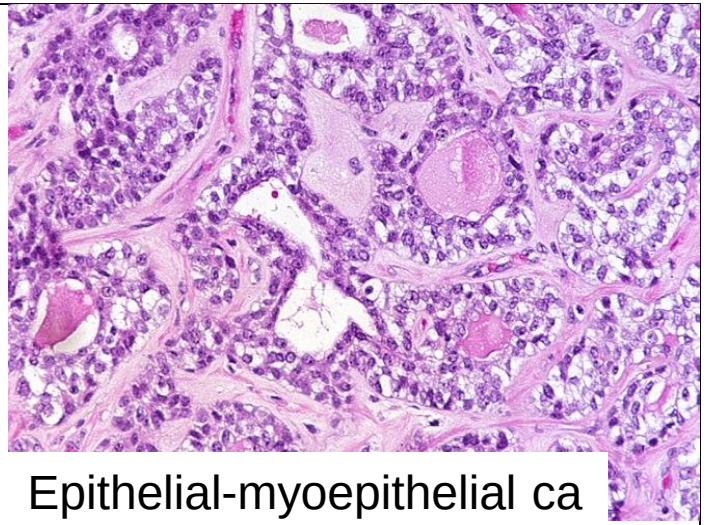
Variable staining with one or more of the following:

- P63/P40
- S100
- Keratin
- Calponin
- Smooth muscle actin
- SOX10

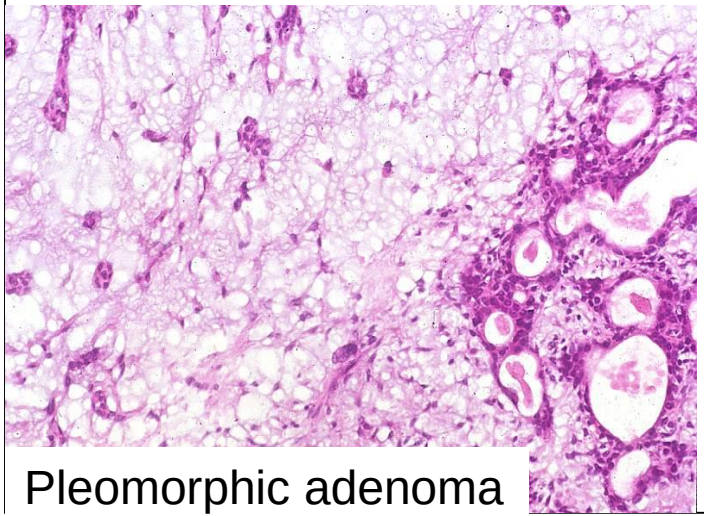




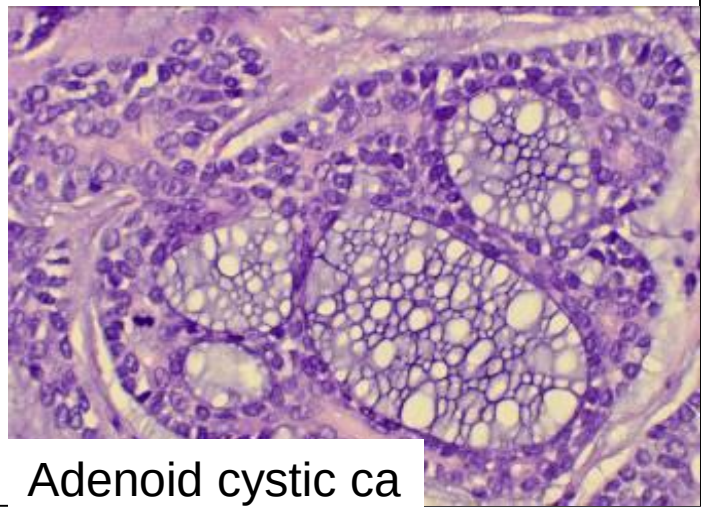
Basal cell adenoma/adenocarcinoma



Epithelial-myoepithelial ca



Pleomorphic adenoma



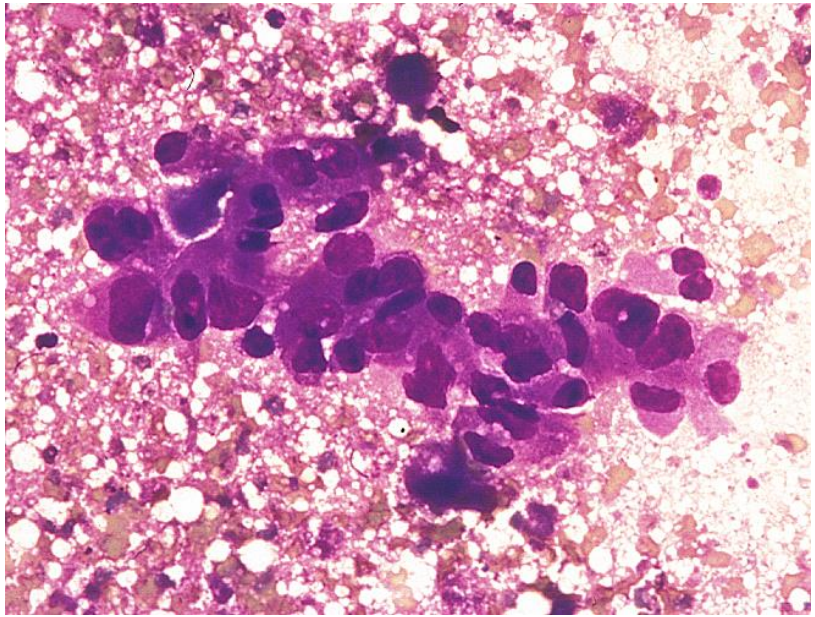
Adenoid cystic ca

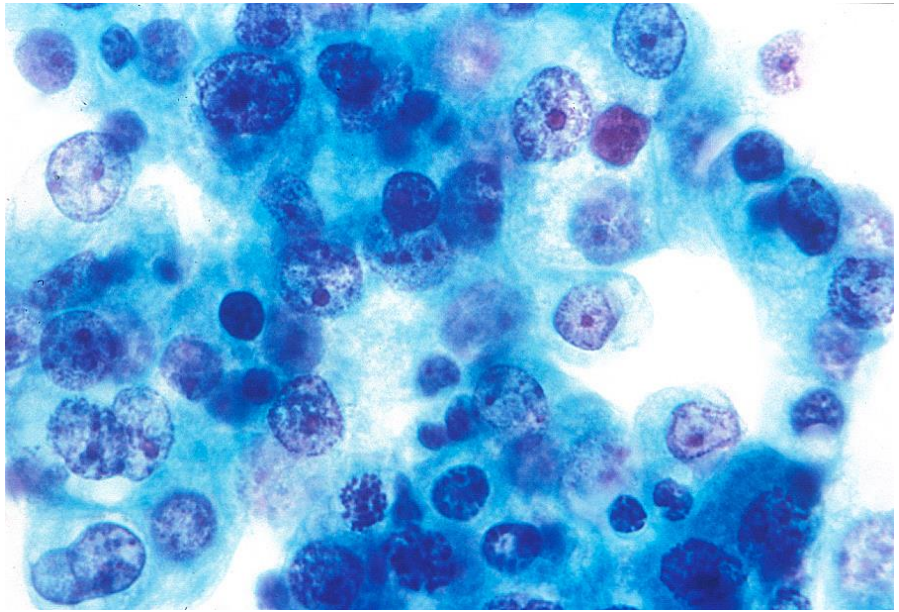
Characteristic immunoprofiles

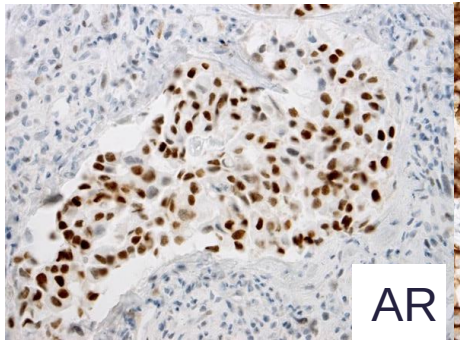
Diagnostic markers not specifically related to defining genetic alterations

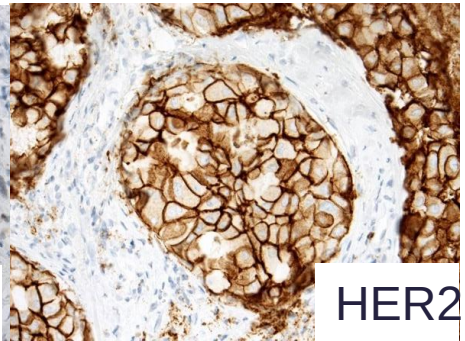
Tumor	IHC Markers
Salivary Duct Carcinoma	**AR+, HER2+(subset)**
Mucoepidermoid Carcinoma	p63+/p40+
Secretory Carcinoma	S100+, mammaglobin+
Polymorphous Adenocarcinoma	p63+/p40-

Salivary duct carcinoma









Surrogate genetic markers

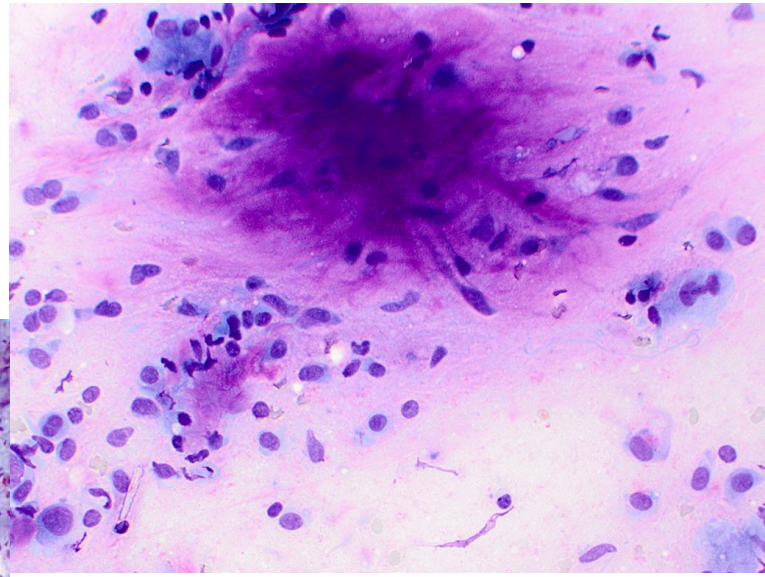
Tumor	Genetic Alteration	Genes Involved	IHC Surrogate Markers
Pleomorphic Adenoma (and Carcinoma ex PA)	Translocation 8q12 Translocation 12q13-15	*PLAG1* *HMGA2*	**PLAG1+** **HMGA2+**
Basal Cell Adenoma	3p22.1 mutation	*CTNNB1* *CYLD*	**β-catenin+**
Adenoid Cystic Carcinoma	t(6;9)(q22-23;p23-24)	*MYB-NFIB*	**MYB+**
Mucoepidermoid Carcinoma	t(11;19)(q21;p13) t(11;15)(q21;q26)	*CRCT1-MAML2* *CRCT3-MAML2*	
Secretory Carcinoma	t(12;15)(p13;q25)	*ETV6-NTRK3*	**Pan-TRK+**
Acinic Cell Carcinoma	t(4;9)(q13;q31)	*NR4A3*	**NR4A3+**
Clear Cell Carcinoma	t(12;22)(q13;q12)	*EWSR1-ATF1*	
Polymorphous Adenocarcinoma	14q12 mutation	*PRKD* family	

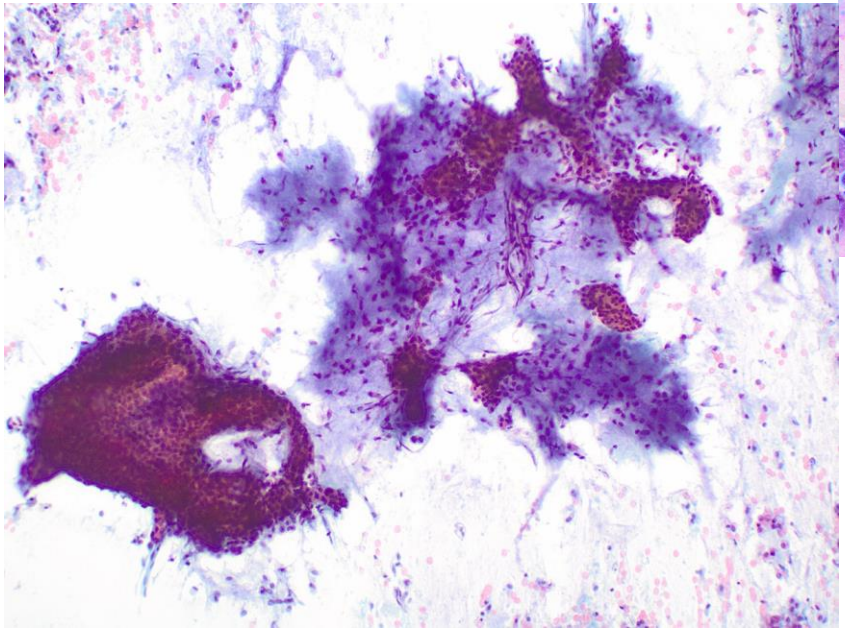
Pleomorphic adenoma

Epithelial cells

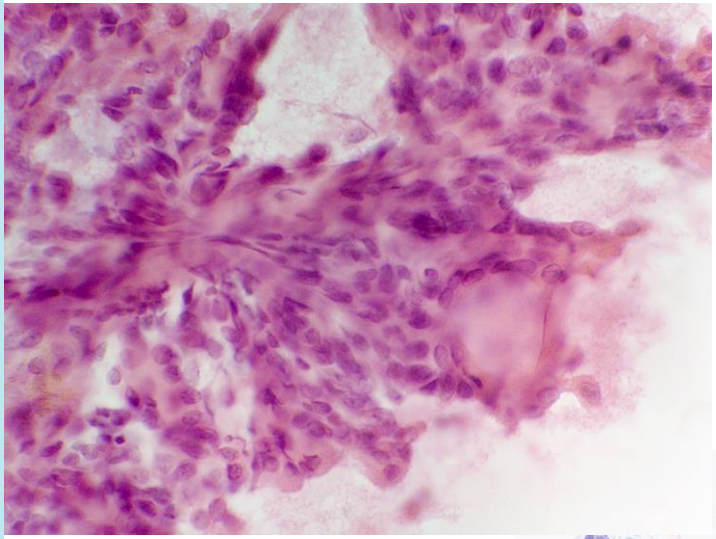
Myoepithelial cells

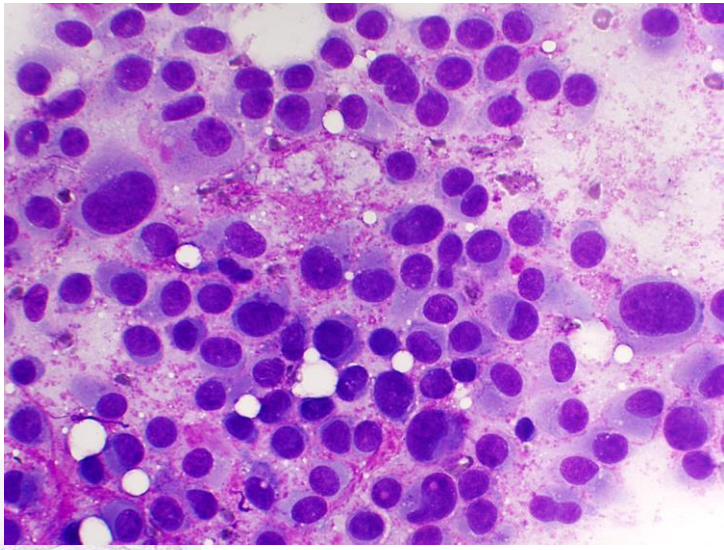
Fibrillary chondromyxoid matrix

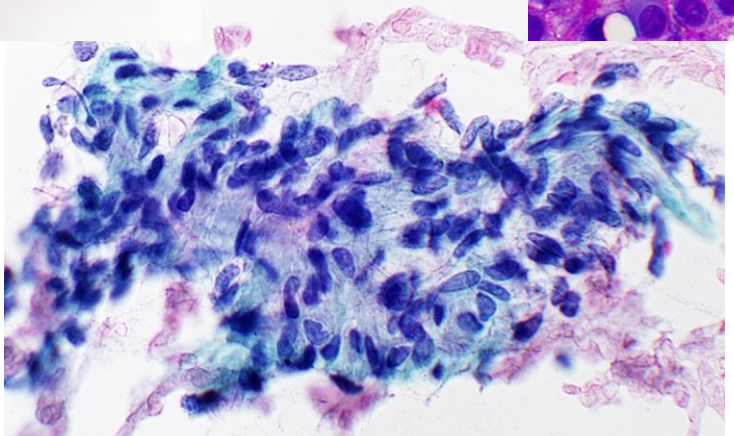




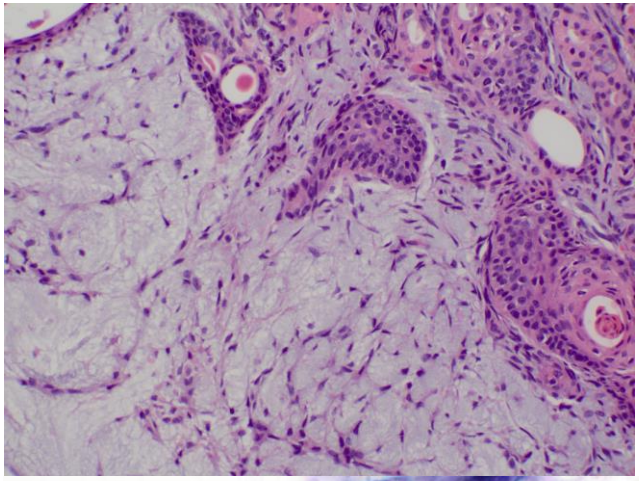
Pleomorphic adenoma

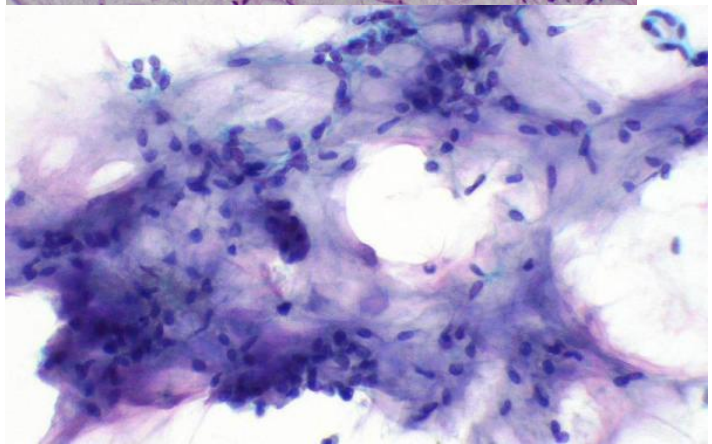






Pleomorphic adenoma





Recurrent translocations:

- 8q12 (*PLAG1* locus) (50-60%)
- 12q13-15 (*HMGA2* locus) (10-15%)
- Can FISH for *PLAG1*, *HMGA2*

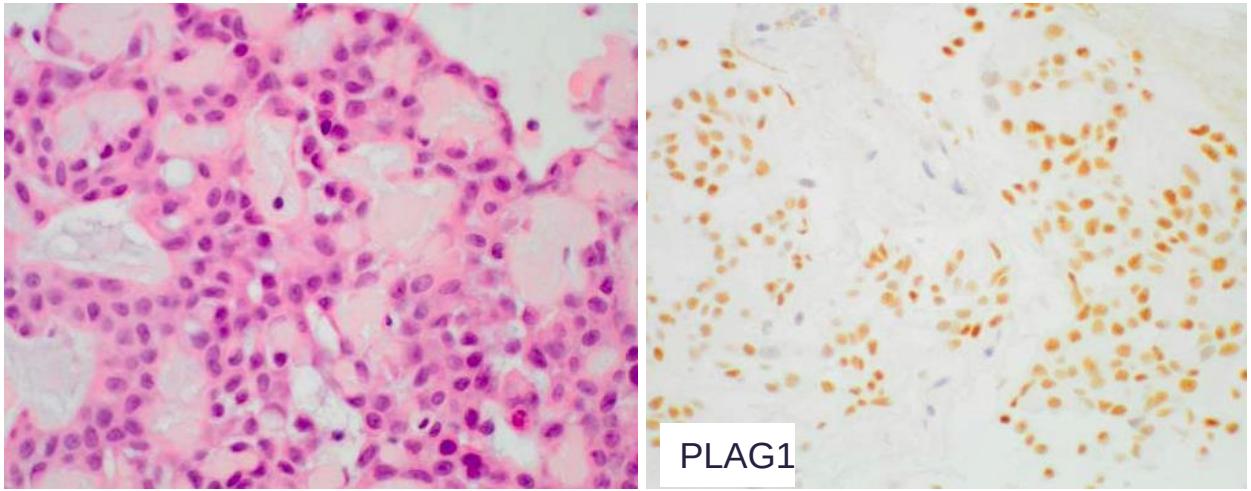
Alterations persist in carcinoma ex PA

- Bahrami et al *Head Neck Pathol* (2012)
- Katabi et al *Hum Pathol* (2015)

PA vs adenoid cystic carcinoma

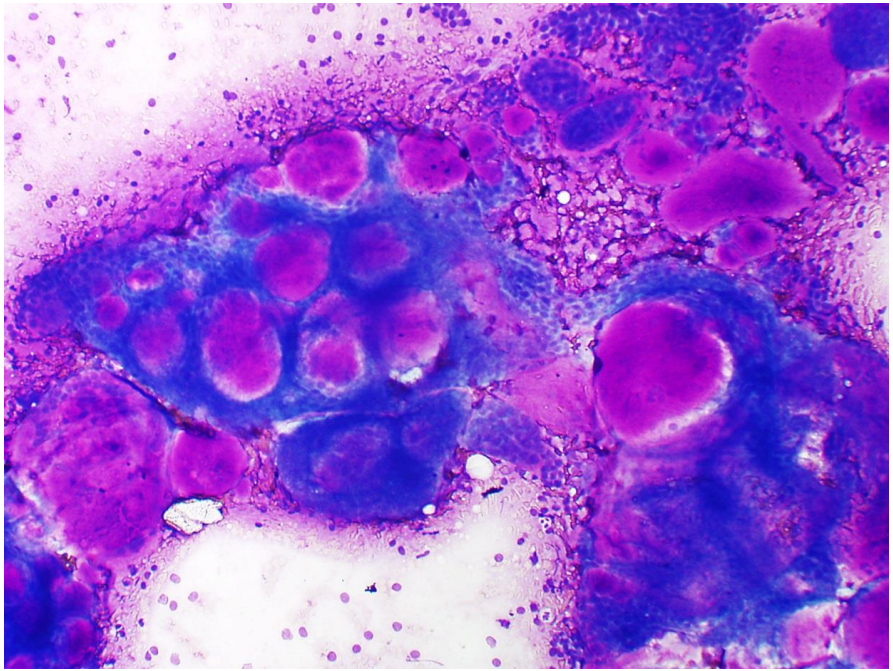
IHC for translocation associated proteins

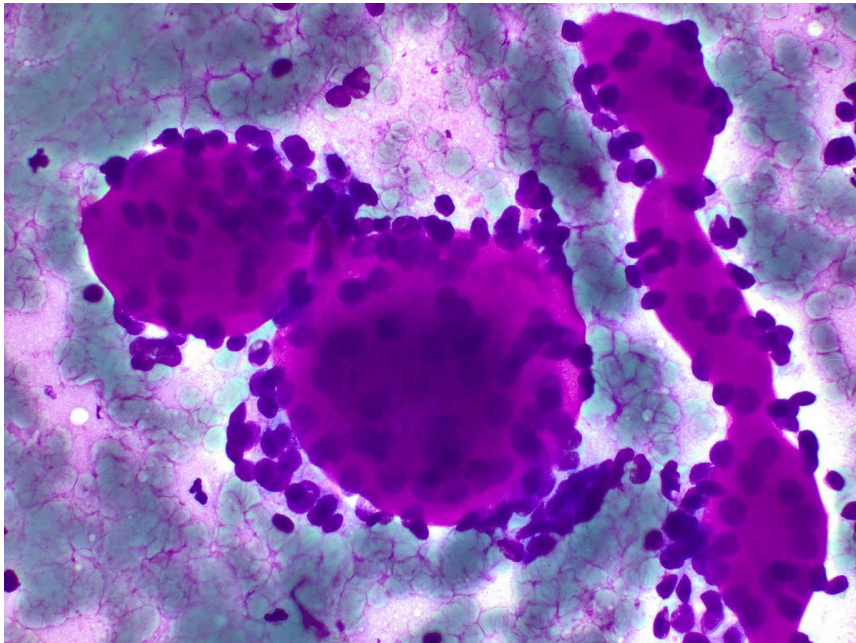
Diagnosis	PLAG1	HMGA2
PA	22/30 (73%)	3/25 (12%)
Adenoid Cystic	0/11 (0%)	0/9 (0%)



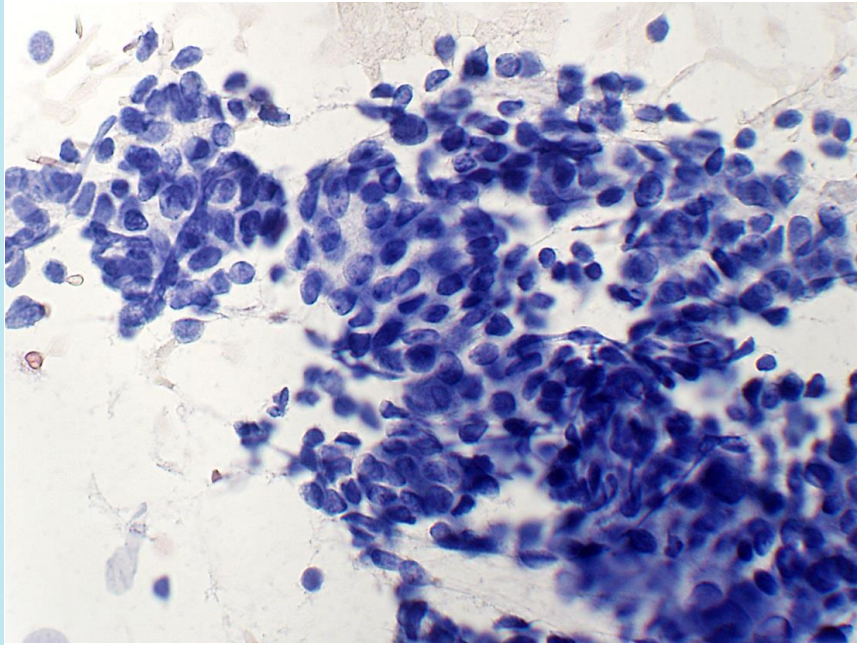
Foo, Jo and Krane *Cancer Cytopathol* (2016)

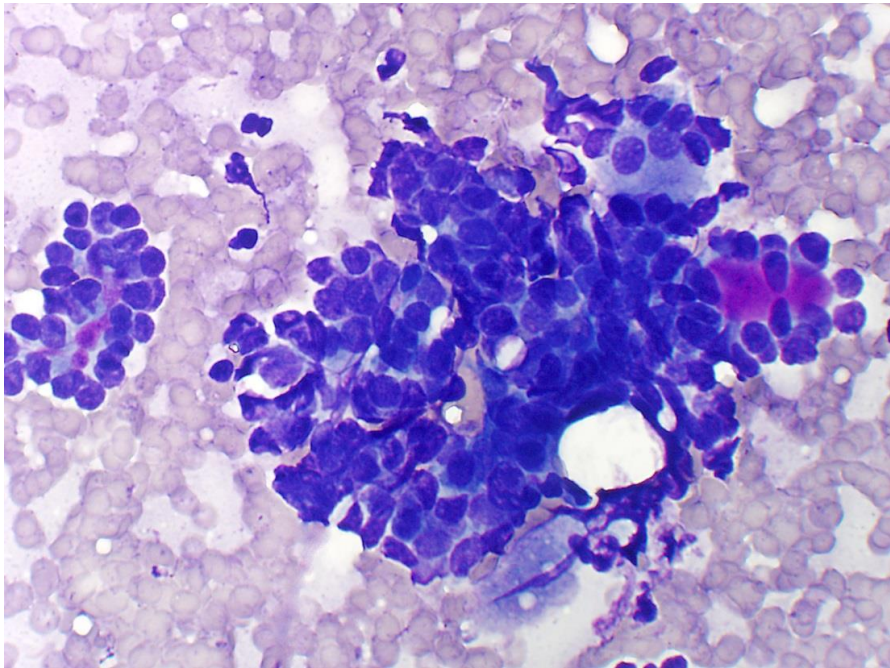
Adenoid cystic carcinoma





Adenoid cystic carcinoma



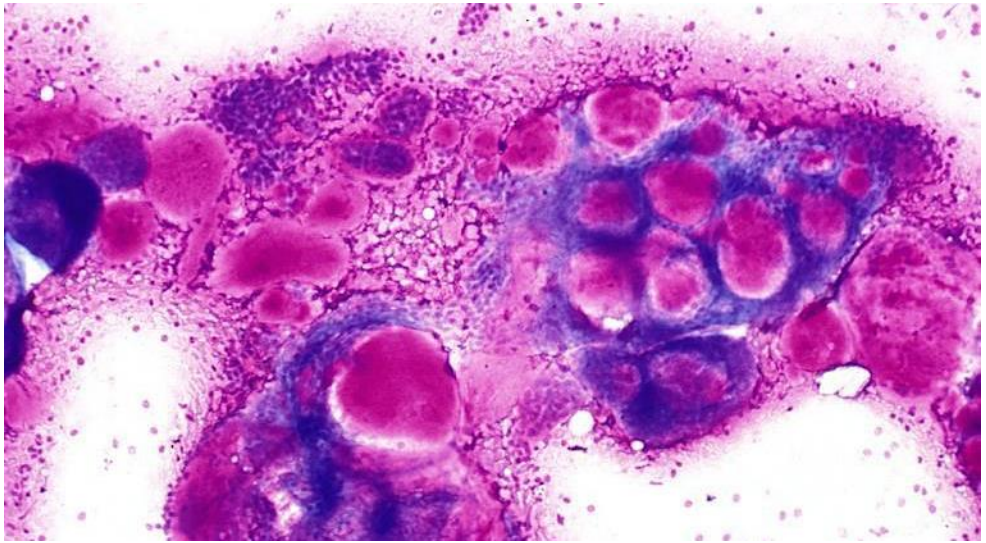


Adenoid cystic carcinoma
Cytogenetics

t(6:9) *MYB* oncogene-*NFIB* transcription factor

- Rarely t(8:9) *MYBL1/NFIB*

>80% of AdCC



Persson et al PNAS (2009)
Persson et al Genes, Chromosomes, and Cancer (2012)

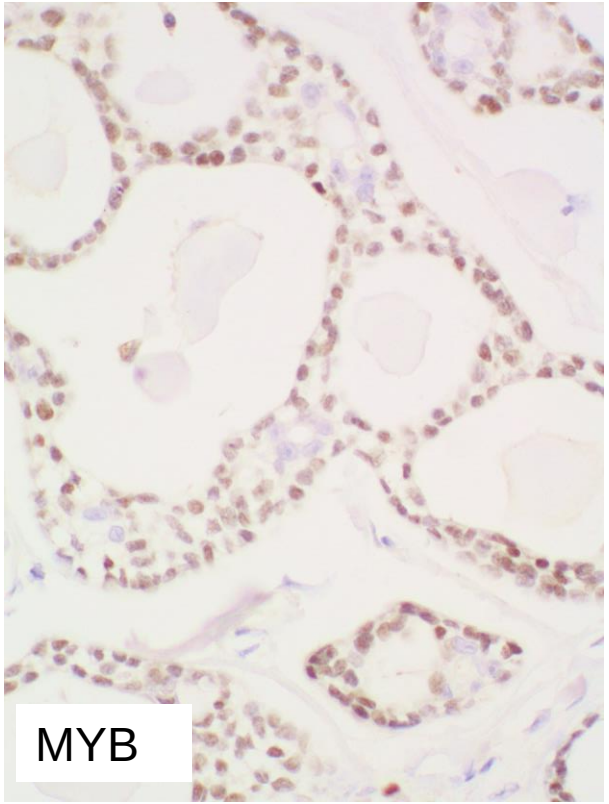
MYB immunohistochemistry

82% AdCC (+)

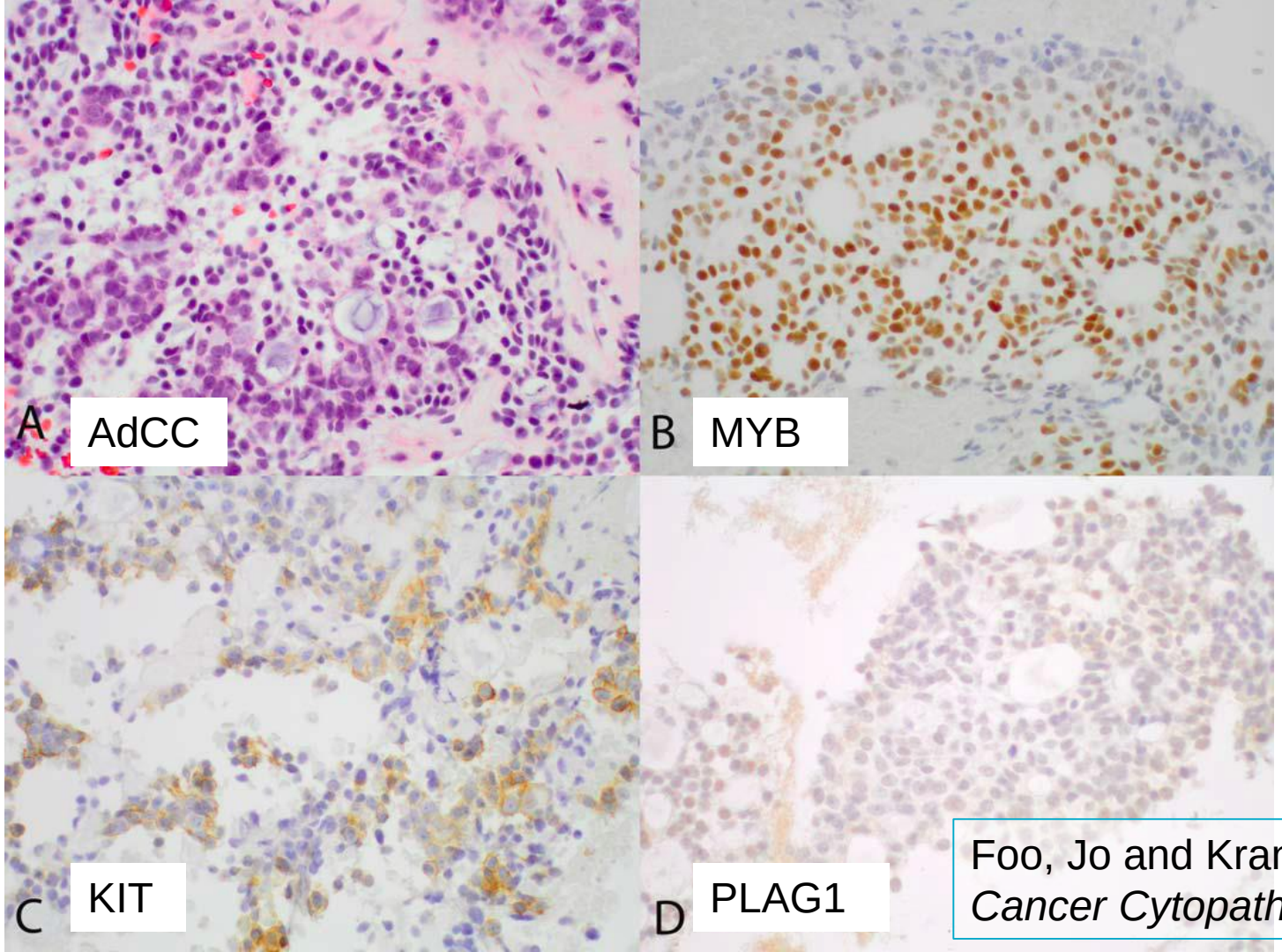
14% non-AdCC tumors tested (+)

- 4 of 5 basaloid SCCs

All non-AdCC tumors were translocation (-)



Brill et al Modern Pathol (2011)



Foo, Jo and Krane
*Cancer Cytopathol (*2016)

PA vs adenoid cystic carcinoma
IHC for translocation associated proteins

PLAG1 or HMGA2+; MYB-, KIT-

- Specific and reasonably sensitive (0.75) for PA

MYB+ and KIT+, PLAG1- and HMGA2-

- Specific for AdCC
- Low sensitivity (0.18)

Foo, Jo and Krane *Cancer Cytopathol* (2016)

FISH/Cytogenetics/NGS

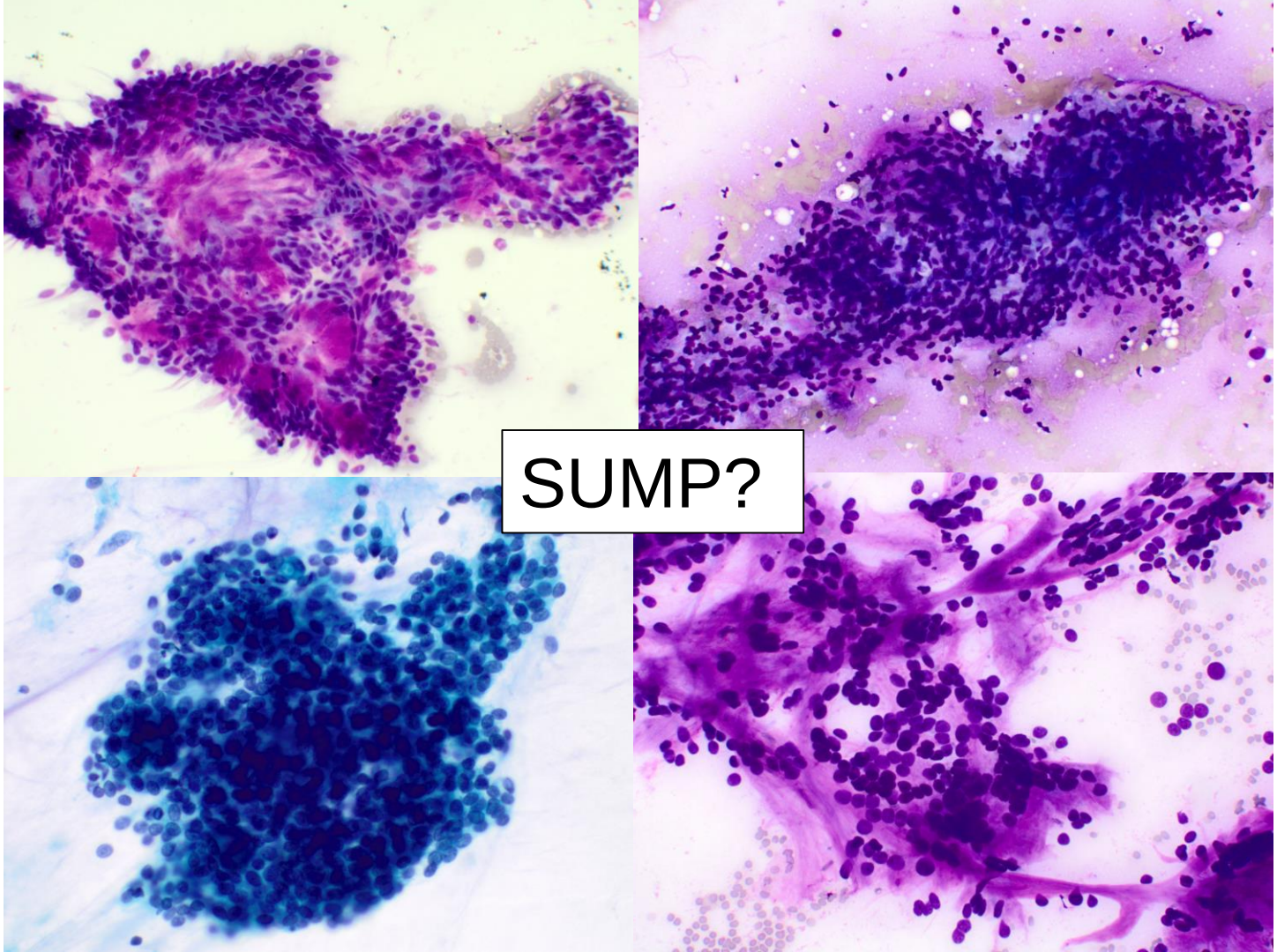
Tumor	Genetic Alteration	Genes Involved	FISH Probe	IHC Markers
Pleomorphic Adenoma (and Carcinoma ex PA)	Translocation 8q12 Translocation 12q13-15	*PLAG1* *HMGA2*	*PLAG1* *HMGA2*	PLAG1 + HMGA2 +
Adenoid Cystic Carcinoma	t(6;9)(q22-23;p23-24)	*MYB-NFIB*	*MYB*	MYB +
Mucoepidermoid Carcinoma	t(11;19)(q21;p13) t(11;15)(q21;q26)	*CRCT1-MAML2* *CRCT3-MAML2*	***MAML2***	p63/p40+
Secretory Carcinoma	t(12;15)(p13;q25)	*ETV6-NTRK3*	*ETV6*	S100+, mammaglobin+ Pan-TRK+
Acinic Cell Carcinoma	t(4;9)(q13;q31)	*NR4A3*	*NR4A3*	NR4A3+
Clear Cell Carcinoma	t(12;22)(q13;q12)	*EWSR1-ATF1*	*EWSR1*	
Polymorphous Adenocarcinoma	14q12 mutation	*PRKD* family		

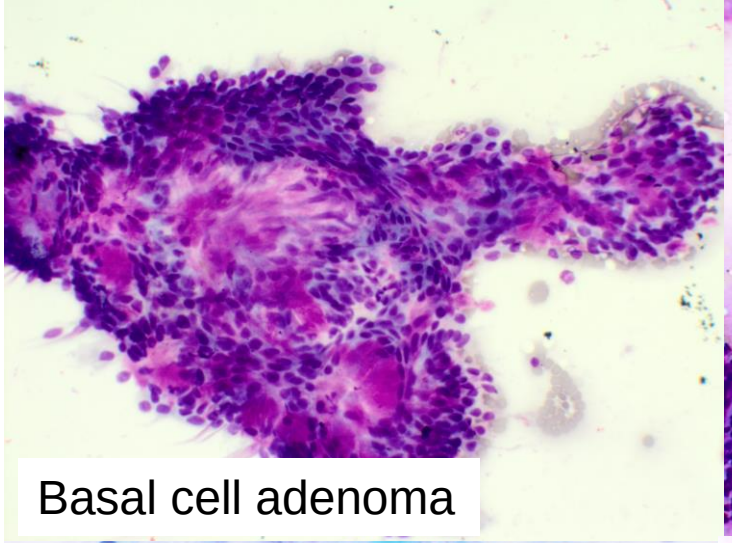
NGS on FNA

The PRKD1 E710D Hotspot Mutation Is Highly Specific in Separating Polymorphous Adenocarcinoma of the Palate From Adenoid Cystic Carcinoma and Pleomorphic Adenoma on FNA

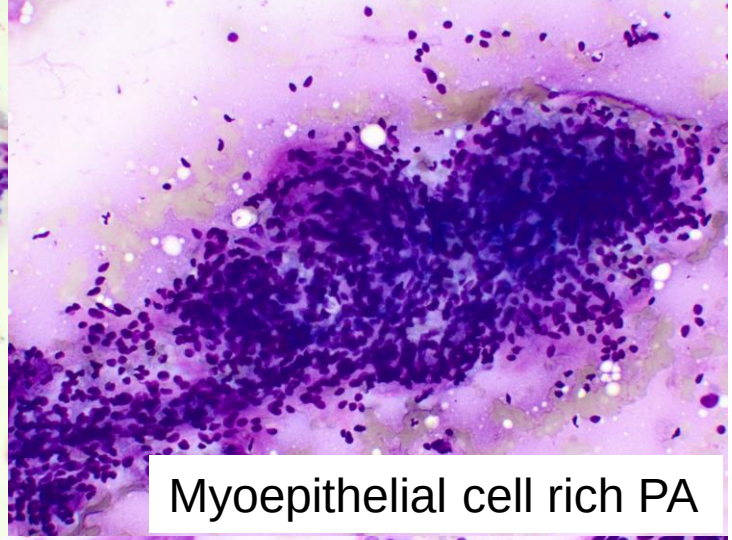
Simon Andreasen, MD [1,2]; Linea Cecilie Melchior, PhD[3]; Katalin Kiss, MD[3]; Justin Avery Bishop, MD[4]; Estrid Høgdall, PhD, DMSc [5]; Morten Grauslund, PhD[3]; Irene Wessel, MD, PhD[2]; Preben Homøe, MD, PhD, DMSc[1]; and Tina Klitmøller Agander, MD, PhD[3]

BACKGROUND: Polymorphous adenocarcinoma (PAC) of the palatal minor salivary glands, previously known as polymorphous low-grade adenocarcinoma, is the second most common intraoral malignant salivary gland carcinoma after adenoid cystic carcinoma (ACC) and carries an excellent prognosis. Unfortunately, PAC demonstrates cytological overlap with 2 other salivary gland tumors frequently encountered in the same location, namely ACC and pleomorphic adenoma (PA). Recently, the protein kinase D1 (*PRKD1*) hotspot mutation E710D was demonstrated to be specific for PAC and to be present in the majority of cases. The objective of the current study was to investigate the value of *PRKD1* hotspot sequencing in identifying PAC in paired fine-needle aspiration (FNA) and surgical specimens from cases of PAC, ACC, and PA. **METHODS:** Paired May-Grunwald-Giemsa-stained FNA and corresponding surgical specimens were collected from 18 PAC cases, 25 ACC cases, and 21 PA cases. Both sets of specimens were subjected to dideoxynucleotide sequencing of *PRKD1* exon 15, including the PRKD1 E710D hotspot. **RESULTS:** Of the PAC cases, approximately 50% demonstrated identical PRKD1 E710D hotspot mutations on the FNA specimen and corresponding surgical specimen. Two ACC specimens had point mutations within the sequenced region in the FNA specimen as well as the surgical specimen, but none were located in the hotspot region. None of the PA cases demonstrated *PRKD1* mutations. The specificity of the *PRKD1* hotspot mutation for identifying PAC among ACC and PA cases was 100% whereas the sensitivity was 50%. **CONCLUSIONS:** The *PRKD1* E710D hotspot mutation is highly specific for identifying PAC on FNA among cases of ACC and PA, whereas the sensitivity is only modest. Alternative *PRKD1* mutations exclude PAC, and are more suggestive of ACC. ***Cancer Cytopathol* 2018;126:275-81.** © *2017 American Cancer Society.*

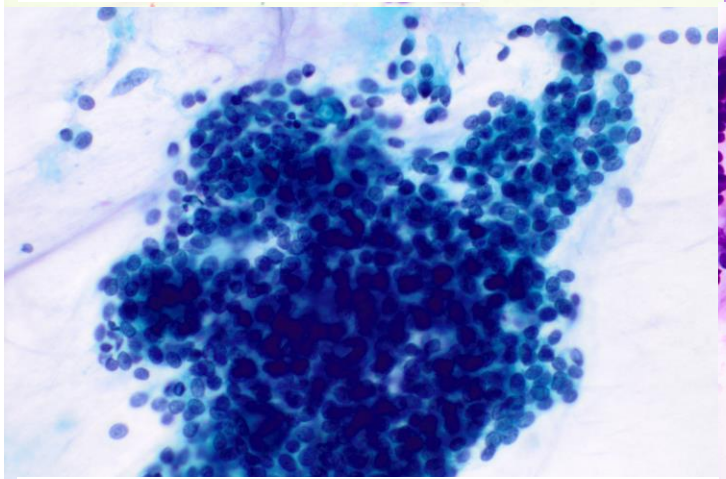

SUMP?



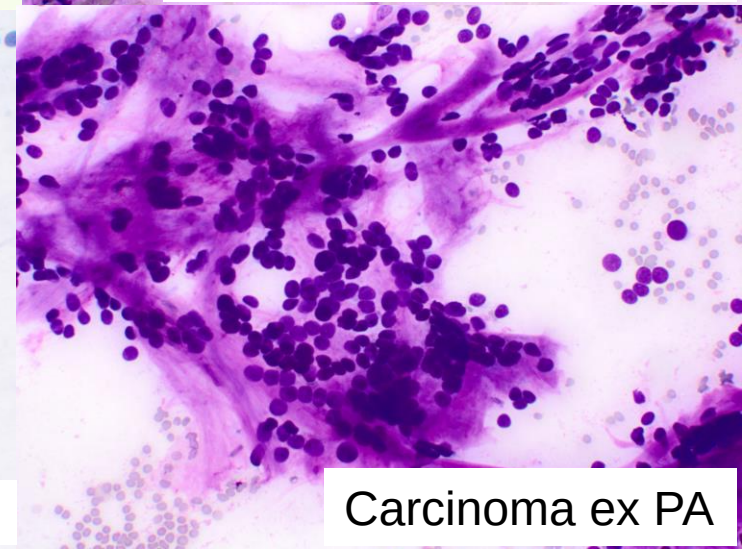
Basal cell adenoma



Myoepithelial cell rich PA



Adenoid cystic carcinoma, solid type



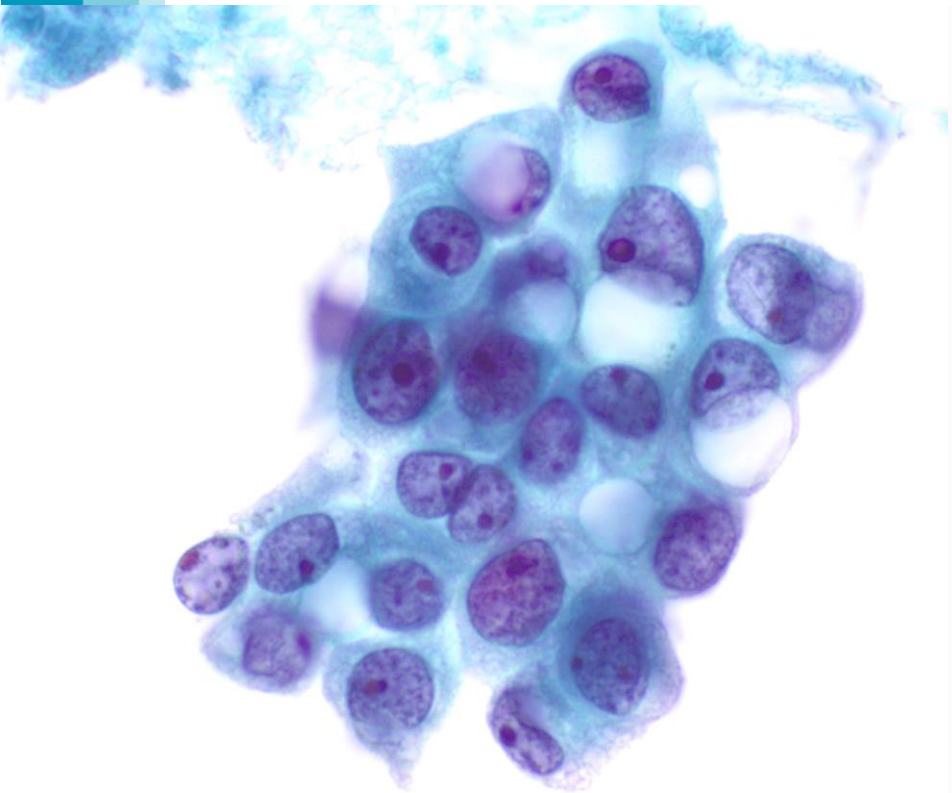
Carcinoma ex PA

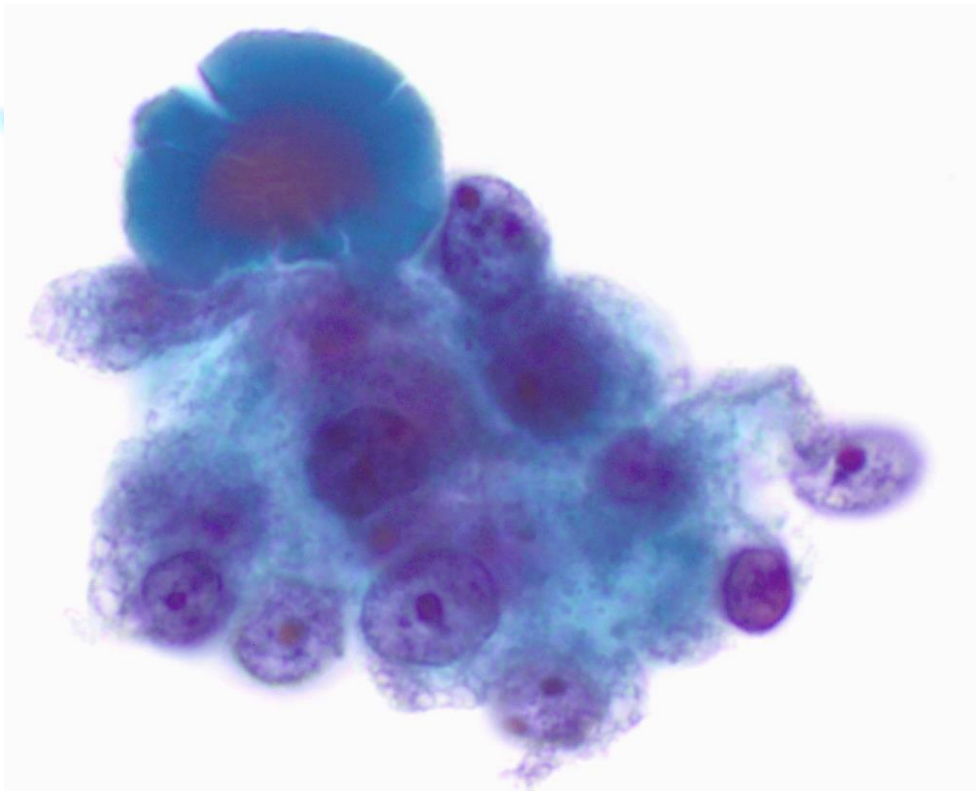
The Milan System for Reporting Salivary Gland Cytopathology

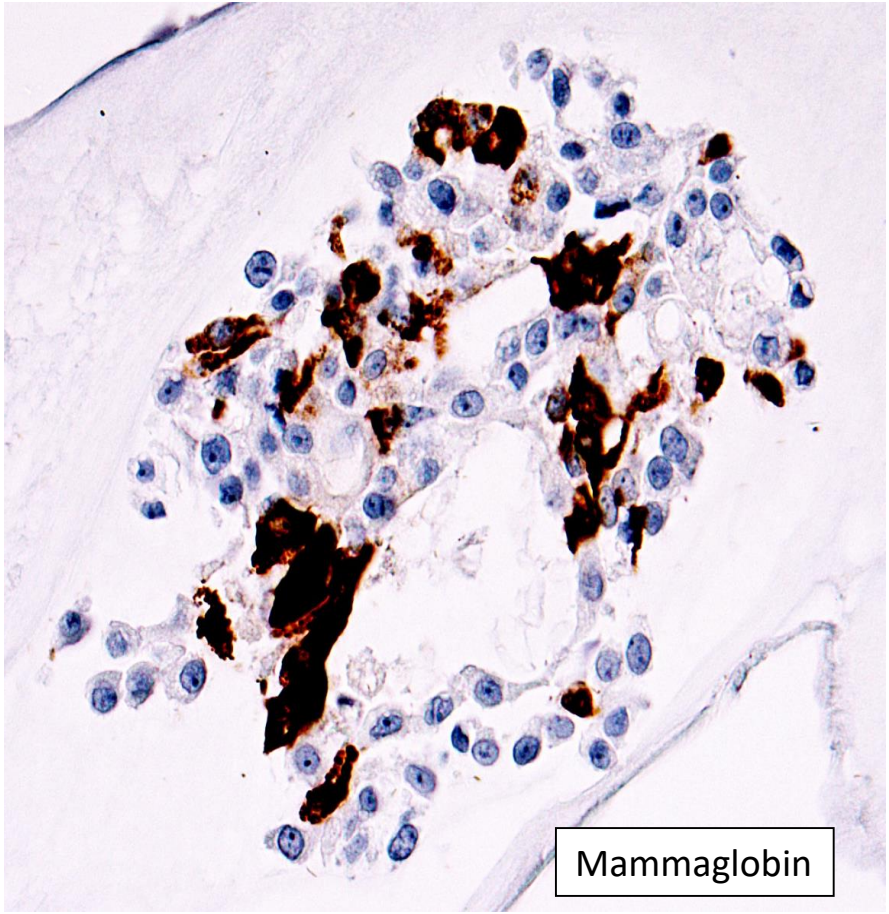
Overview of Terminology and Reporting

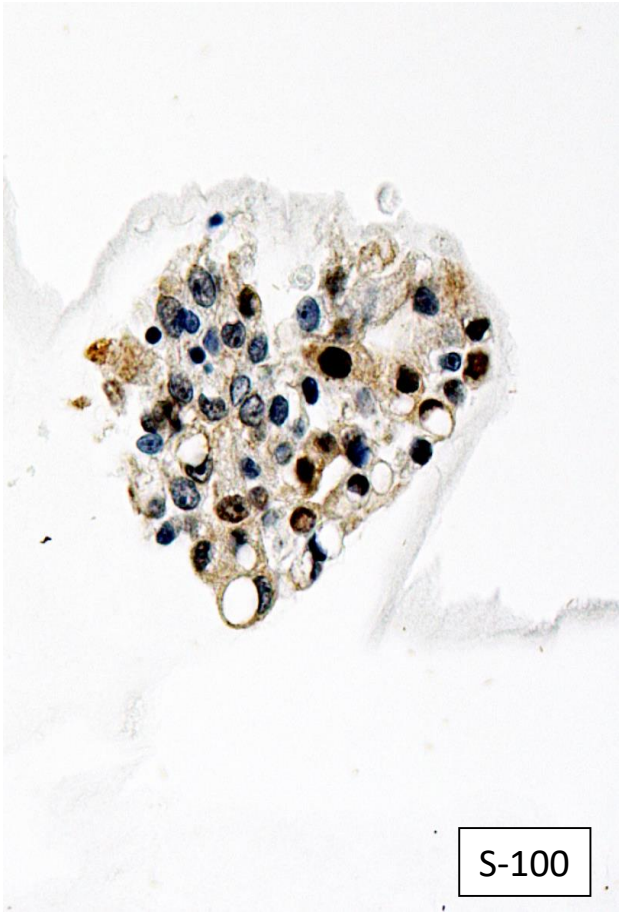
Diagnostic Category	ROM	Management
I. Non-Diagnostic	25%	Clinical and radiologic correlation/ repeat FNA
II. Non-Neoplastic	10%	Clinical follow-up and radiologic correlation
III. Atypia of Undetermined Significance (AUS)	20%	Repeat FNA or surgery
IV. Neoplasm		
A. Benign	<5%	Surgery or Clinical F/U
B. Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)	35%	Surgery
V. Suspicious for Malignancy	60%	Surgery
VI. Malignant	90%	Surgery

Case:
?High-grade mucoepidermoid carcinoma

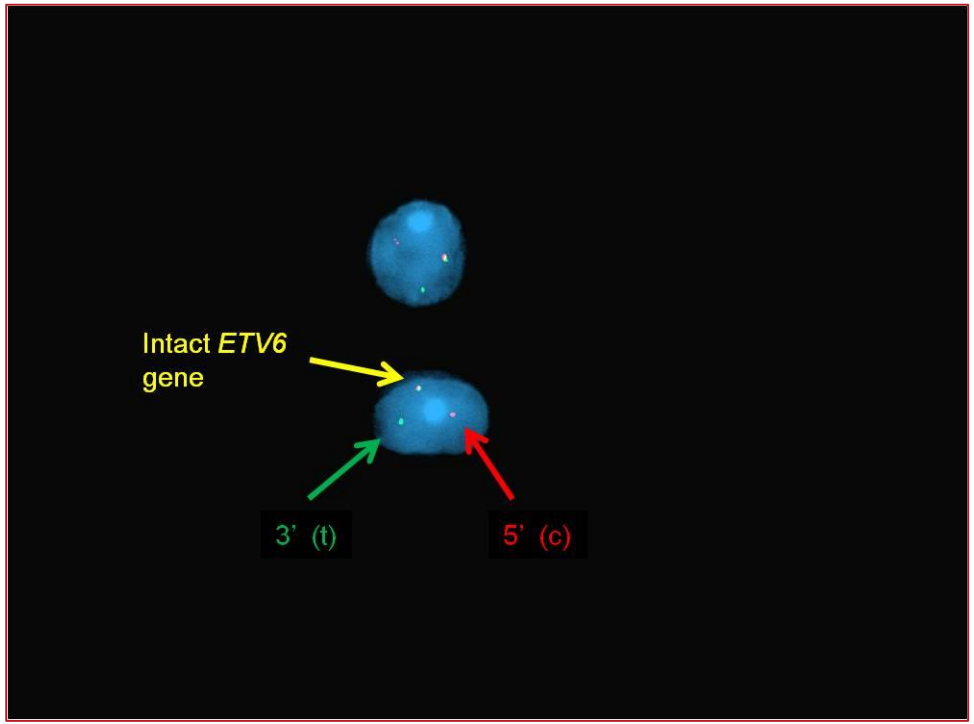





Mammaglobin

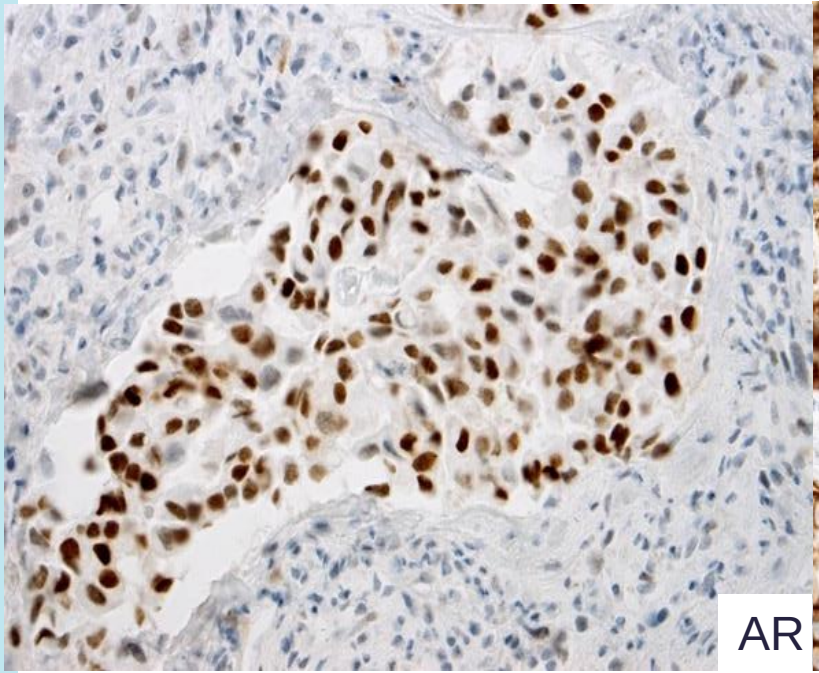

S-100

(Mammary analogue) Secretory carcinoma

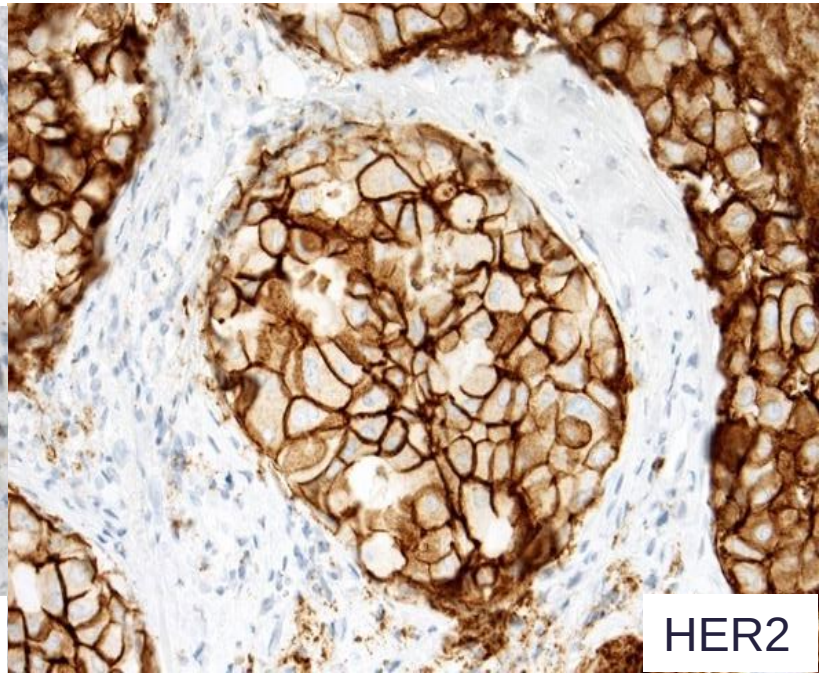


Salivary duct carcinoma
Therapeutic as well as diagnostic utility

Anti-androgenic agents



Herceptin



Salivary Duct Carcinoma

- Subset are SDC ex PA
  - Distinct molecular profile
    - More likely HER2+
  - PLAG1 or HMGA2 expression if rearrangement present
  - Precursor PA may not be sampled and/or focal

What should our expectations be for ancillary testing of salivary gland FNA?

Benefits

Minimizes diagnostic uncertainty

Refines risk stratification

Optimizes patient management

Promotes clinician confidence

Obstacles

ROSE availability

Technical expertise

Time

Financial costs

Final thoughts on ancillary testing

Milan system recognizes the value of ancillary techniques, but is not proscriptive

Provides a framework for deciding what makes sense in your lab while balancing

- Optimal performance of salivary gland FNA
- Clinical expectations and needs
- Available resources

One year in, where does Milan system stand?

Category use

11 retrospective studies since 2018

Diagnostic Category	Median	Range
I. Non-Diagnostic	13.5%	2.8-23%
II. Non-Neoplastic	23%	7-42%
III. Atypia of Undetermined Significance (AUS)	3.2%	2-10.8%
IV. Neoplasm		
A. Benign	36.9%	27.9-51.4%
B. Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)	5.4%	1.3-14.2%
V. Suspicious for Malignancy	2.4%	1.2-14%
VI. Malignant	9.4%	2.5-17%

Kala et al
Chen et al
Sadullahoglu et al
Wu et al
Karuna et al
Pujani et al
Song et al
Vallonthaiel et al
Viswanathan et al
Montezuma et al
Thiryayi et al

Interobserver reproducibility

TABLE 2 Summary of agreement by category for the Milan system

MilanCategory	Description	kappa
1	Non-diagnostic	0.45
2	Non-neoplastic	0.29
3	Atypia of undetermined significance	0.17
4A	Neoplasm, Benign	0.71
4B	Neoplasm of uncertain malignant potential	0.15
5	Suspicious for malignancy	0.15
6A	Malignant, low grade	0.20
6B	Malignant, high grade	0.72
Average		0.42

3 observers, reviewing 408 cases

Layfield et al *Diagn Cytopathol* (2019)

ROM in the real world

- All retrospective to date
- Meta-analysis of 92 studies pre-Milan (Farahani and Baloch)
- 4453 FNAs in 17 studies post-Milan (since 2018)

Diagnostic Category	Anticipated ROM	Meta-analysis ROM	Post-Milan ROM
I. Non-Diagnostic*	25%	20.6%	16.4%
II. Non-Neoplastic*	10%	8.9%	7.6%
III. Atypia of Undetermined Significance (AUS)*	20%	37.3%	33.5%
IV. Neoplasm			
A. Benign	<5%	5.4%	3.2%
B. Salivary Gland Neoplasm of Uncertain Malignant Potential (SUMP)	35%	31.5%	36.4%
V. Suspicious for Malignancy	60%	61.5%	84%
VI. Malignant	90%	89.1%	96.8%

Mishra et al
Kala et al
Chen et al
Choy et al
Sadullahoglu et al
Wu et al
Mazzola et al
Karuna et al
Jaiswal et al
Park et al
Savant et al
Pujani et al
Song et al
Vallonthaiel et al
Viswanathan et al
Layfield et al
Thiryayi et al

Summary

The Milan System for Reporting Salivary Gland Cytopathology:

1. **Standardizes reporting terminology**
2. **Provides risk stratification**
3. **Guides clinical management**

Ancillary testing can improve performance of, and increase confidence in, The Milan System

Early data are largely encouraging regarding its use

Pearls of Pathology

Use Milan System terminology

Consider ancillary testing to:

1. **Resolve diagnostic uncertainty**
2. **Change the Milan System category**
3. **Improve risk stratification**
4. **Guide clinical management**

References

Faquin WC and Rossi ED (Eds.). The Milan System for Reporting Salivary Gland Cytopathology. Cham, Switzerland: Springer International Publishing AG; 2018.

Jo VY and Krane JF. Ancillary Testing in Salivary Gland Cytology: A Practical Guide. Cancer Cytopathol 2018;126:602-17.