WHO Classification of Lung carcinoma and biomarkers

Aim

- Basic overview of classification
  - Emphasis on the importance of the malignant types
  - IHC profile and biomarkers associated with each type
  - Unique characteristics
- Biomarkers
  - Essential molecular biomarkers in Lung cancer
  - Will just touch PD-L1 testing

WHO classification

- Epithelial tumours
  - Papillomas
    - Bronchial papillomas
  - Adenomas
    - Sclerosing pneumocytoma
    - Alveolar adenoma
    - Papillary adenoma of the lung
    - Bronchiolar adenoma
    - Mucinous cystadenoma of the lung
    - Mucous gland adenoma of the lung
  - Precursor glandular lesions
    - Atypical adenomatous hyperplasia of the lung
    - Adenocarcinoma in situ of the lung
  - Adenocarcinomas
    - Minimally invasive adenocarcinoma of the lung
    - Invasive non-mucinous adenocarcinoma of the lung
    - Invasive mucinous adenocarcinoma of the lung
    - Colloid adenocarcinoma of the lung
    - Fetal adenocarcinoma of the lung
    - Enteric-type adenocarcinoma of the lung
  - Squamous precursor lesions
    - Squamous dysplasia and carcinoma in situ of the lung
  - Squamous cell carcinomas
    - Squamous cell carcinoma of the lung
    - Lymphoepithelial carcinoma of the lung
  - Large cell carcinomas
    - Large cell carcinoma of the lung
  - Adenosquamous carcinoma
    - Adenosquamous carcinoma of the lung
  - Sarcomatoid carcinomas
    - Pleomorphic carcinoma of the lung
    - Pulmonary blastoma
    - Carcinosarcoma of the lung
  - Other epithelial tumours
    - NUT carcinoma of the lung
    - Thoracic SMARCA4-deficient undifferentiated tumour
  - Salivary gland-type tumours
    - Pleomorphic adenoma of the lung
    - Adenoid cystic carcinoma of the lung
    - Epithelial-myoepithelial carcinoma of the lung
    - Mucoepidermoid carcinoma of the lung
    - Hyalinizing clear cell carcinoma of the lung
    - Myoepithelioma and myoepithelial carcinoma of the lung
  - Lung neuroendocrine neoplasms
    - Precursor lesion
      - Diffuse idiopathic pulmonary neuroendocrine cell hyperplasia
    - Neuroendocrine tumours
      - Carcinoid/neuroendocrine tumour of the lung
    - Neuroendocrine carcinomas
      - Small cell lung carcinoma
      - Large cell neuroendocrine carcinoma of the lung
  - Tumours of ectopic tissues
    - Melanoma of the lung
    - Meningioma of the lung
  - Mesenchymal tumours specific to the lung
    - Pulmonary hamartoma
    - Pulmonary chondroma
    - Diffuse pulmonary lymphangiomatosis
    - Pleuropulmonary blastoma
    - Pulmonary artery intimal sarcoma
    - Congenital peribronchial myofibroblastic tumour
    - Primary pulmonary myxoid sarcoma with EWSR1-CREB1 fusion
    - PEComatous tumours
      - Lymphangioleiomyomatosis PEComa of the lung
  - Haematolymphoid tumours
    - Haematolymphoid tumours of the lung: Introduction
    - MALT lymphoma of the lung
    - Pulmonary diffuse large B-cell lymphoma
    - Lymphomatoid granulomatosis of the lung
    - Intravascular large B-cell lymphoma of the lung
    - Pulmonary Langerhans cell histiocytosis
    - Pulmonary Erdheim-Chester disease

Too long!

Simplified classification

- **Benign**
  - Papillomas
  - Adenomas
  - Mesenchymal tumors
  - PEComatous tumors
- **Precursor lesions**
  - Atypical adenomatous hyperplasia
  - Adenocarcinoma-in-situ
  - Squamous dysplasia
  - NEC hyperplasia
- **Malignant**
  - Adenocarcinoma
  - Squamous cell carcinoma
  - Adenosquamous cell carcinoma
  - Large cell carcinoma
  - Sarcomatoid carcinoma
  - Neuroendocrine tumors
    - Small cell carcinoma
    - Large cell neuroendocrine carcinoma
  - Other epithelial tumors
  - Salivary gland type carcinoma
  - Hematolymphoid tumors
  - Tumors from ectopic sites

Malignant tumors of the lung

Adenocarcinoma
- Minimally invasive
- Invasive non-mucinous
- Invasive mucinous
- Colloid
- Fetal

Squamous cell carcinoma
- Squamous cell carcinoma
- Lymphoepithelial carcinoma

Adenosquamous carcinoma

Large cell carcinoma

Sarcomatoid carcinoma
- Pleomorphic carcinoma
- Pulmonary blastoma
- Carcinosarcoma

Salivary gland type
- Adenoid cystic carcinoma
- Epithelial-myoepithelial carcinoma
- Hyalinizing clear cell carcinoma
- Myoepithelial carcinoma

Neuroendocrine
- Carcinoid
- Small cell carcinoma
- Large cell neuroendocrine carcinoma

Other epithelial tumors
- NUT carcinoma
- SMARCA4 deficient undifferentiated tumor

Sarcoma

Hematolymphoid

Ectopic

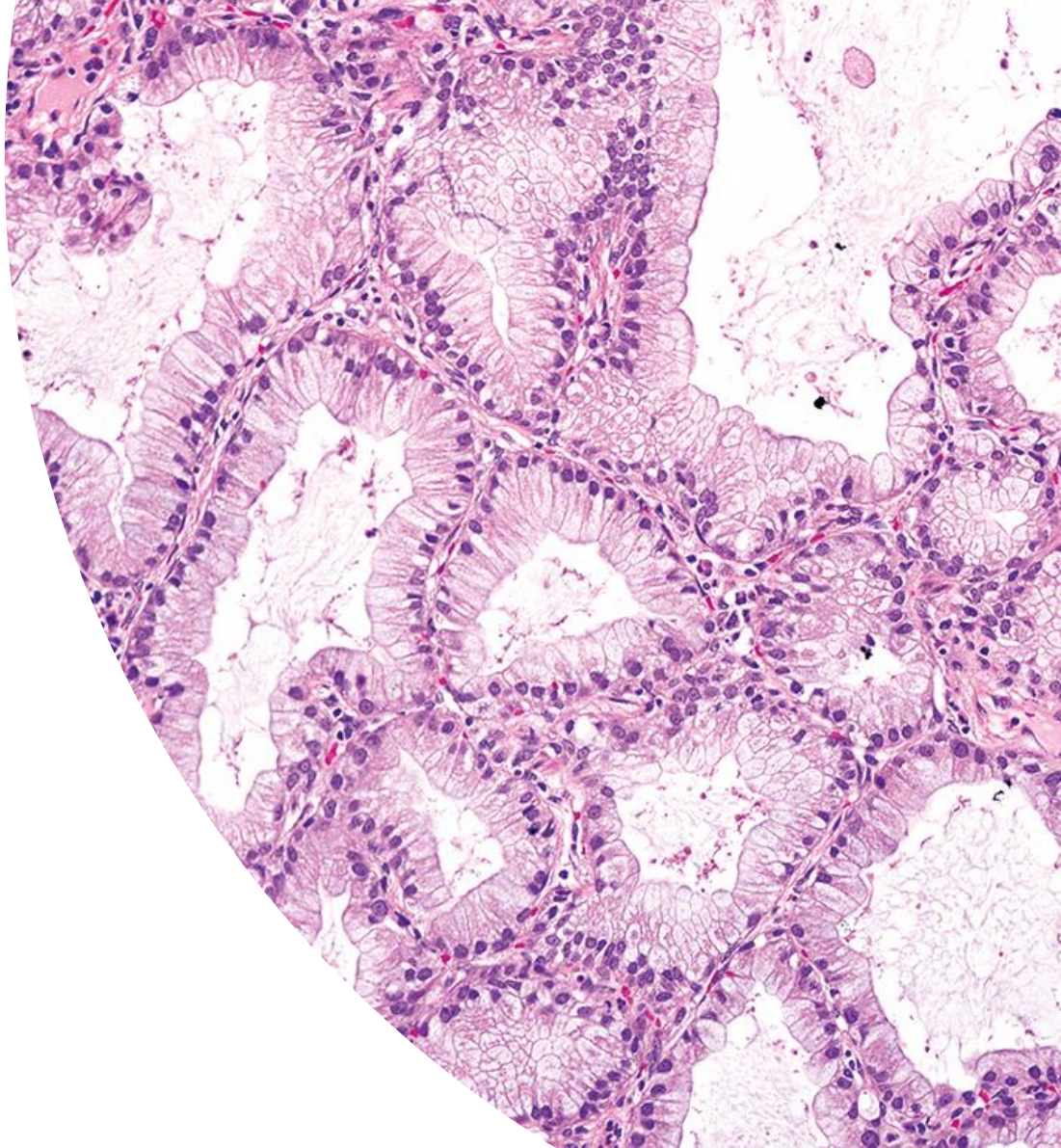
Tissue needed to classify

- Biopsy
  - Usually small biopsy (FNA, Core)
  - Resection

Adenocarcinoma

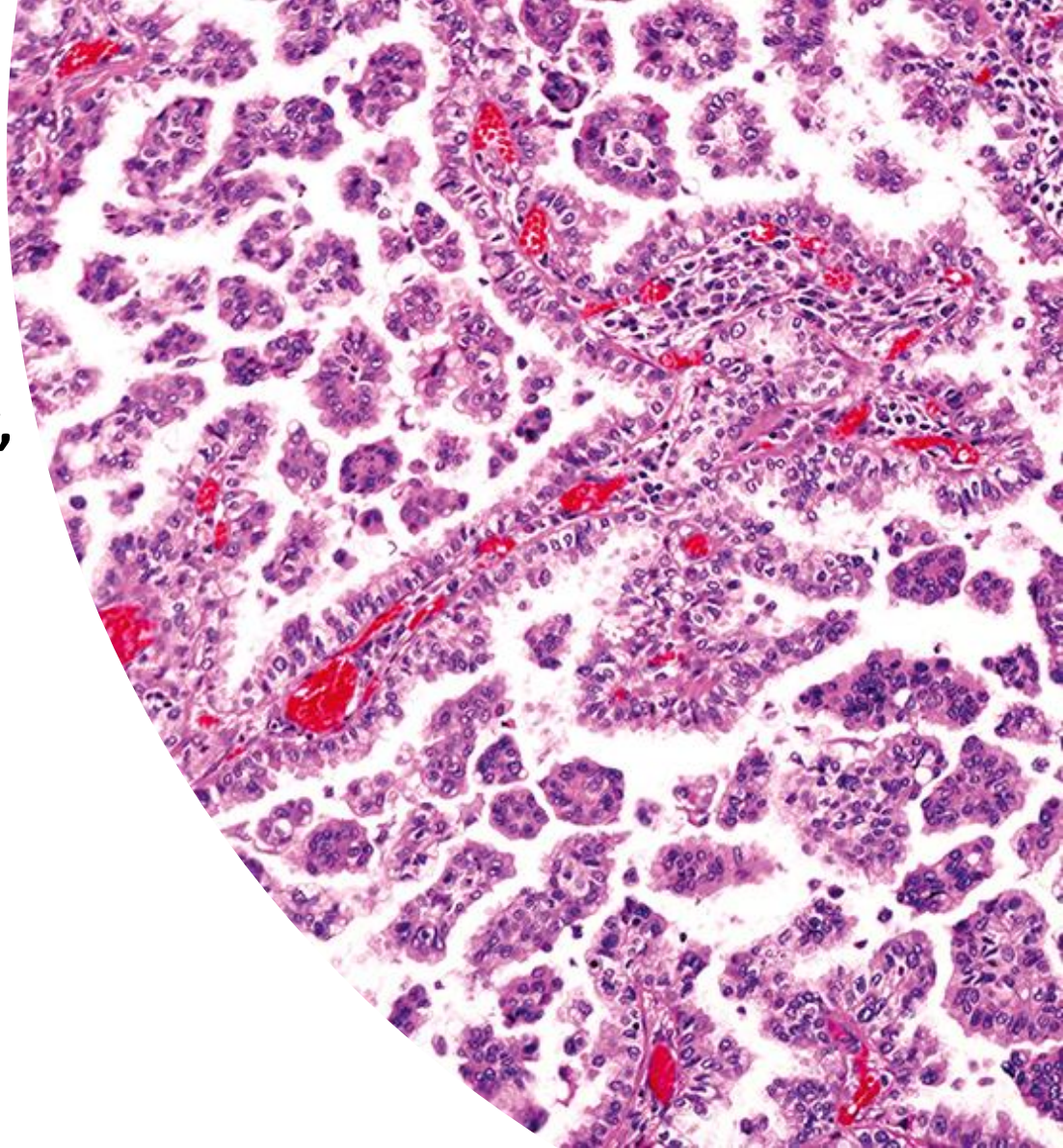
Minimally invasive adenocarcinoma

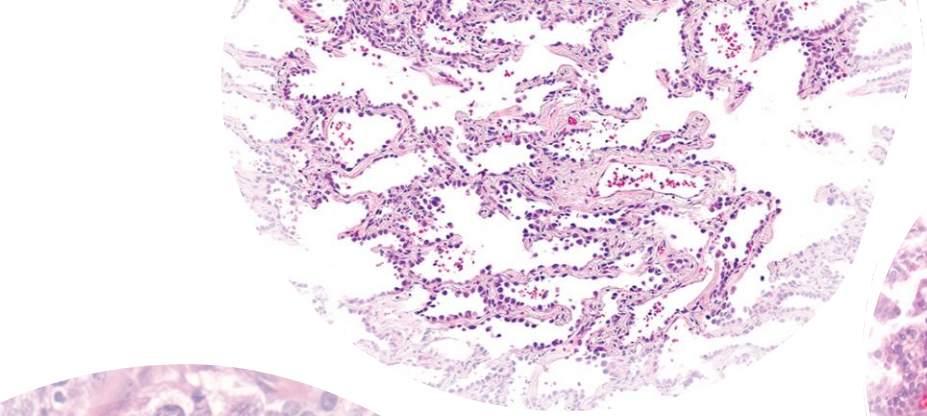
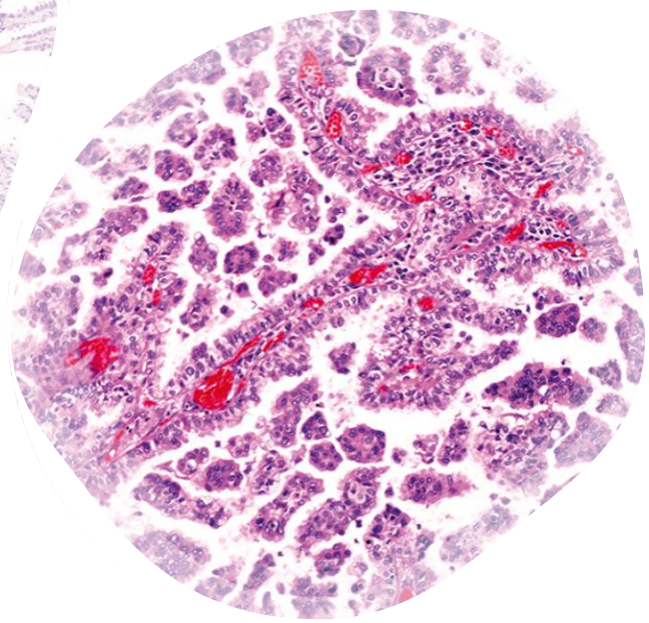
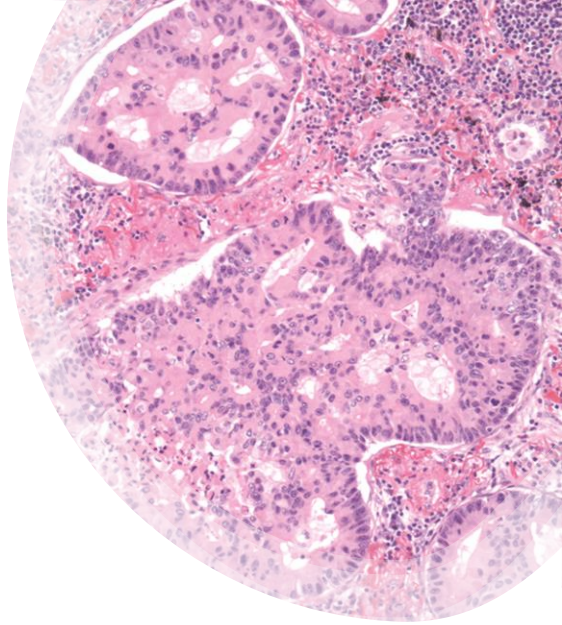
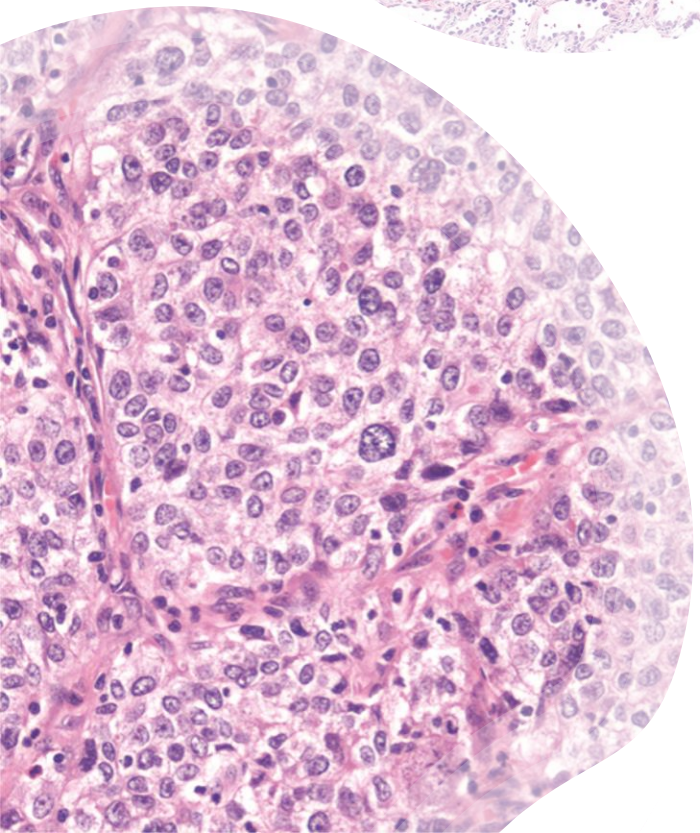
- **Small (≤ 30 mm), Solitary**
- **Predominantly lepidic pattern**
- **≤ 5 mm invasion.**
- **Requires a resection specimen**
- **Mucinous or non-mucinous**
- **100% survival**



Invasive non-mucinous carcinoma

- **Variety of patterns:**
  - **Lepidic, acinar, micropapillary, Solid, mucinous etc.**
- **Invasive**
- **TTF1 and Napsin A positive**
- **Targetable mutations**
  - ***EGFR, ALK, ROS1, BRAF, MET, RET, NTRK, KRAS p.G12C***
- ***ERBB2 mutations often positive***

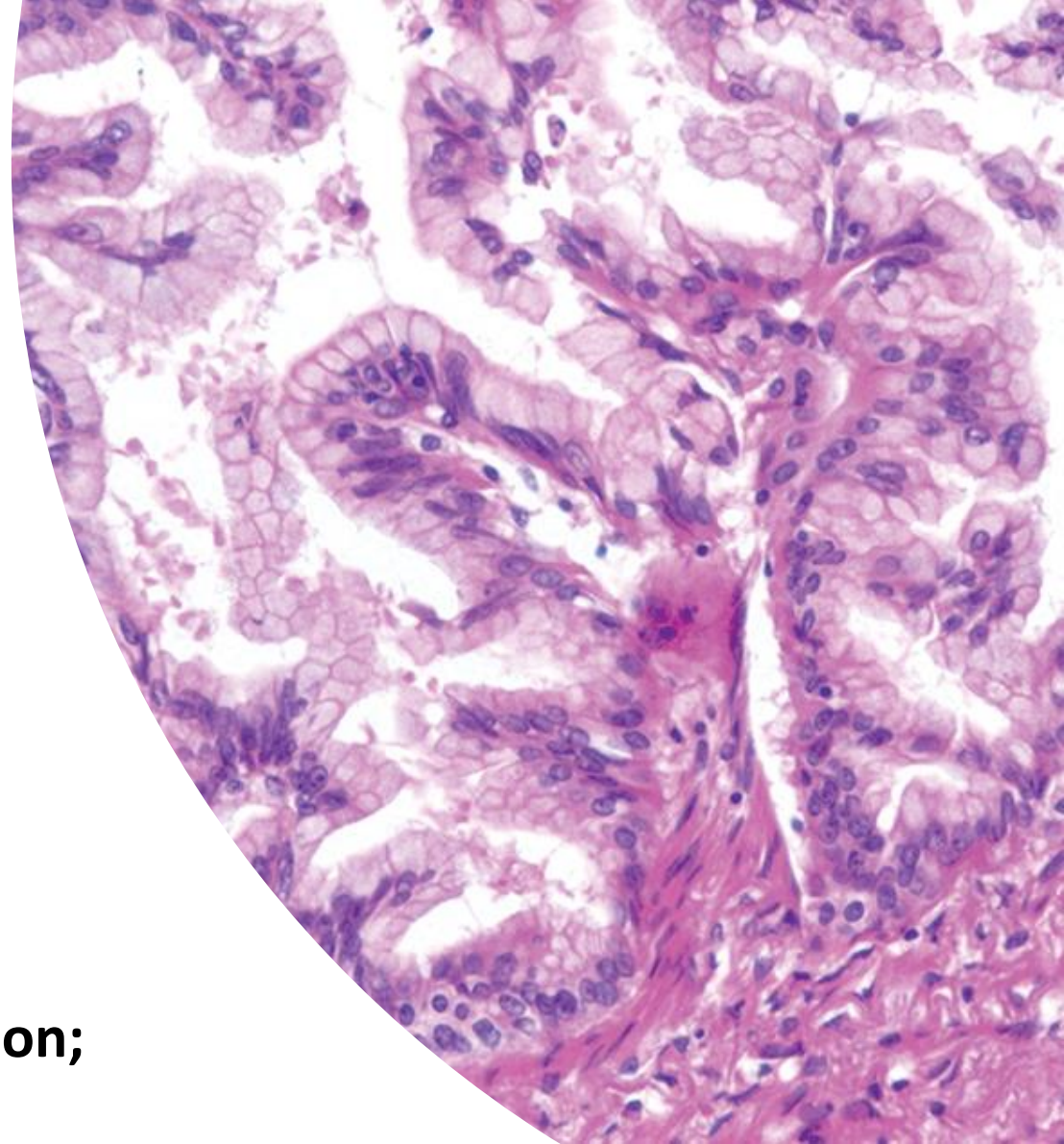


Invasive non-mucinous carcinoma, patterns

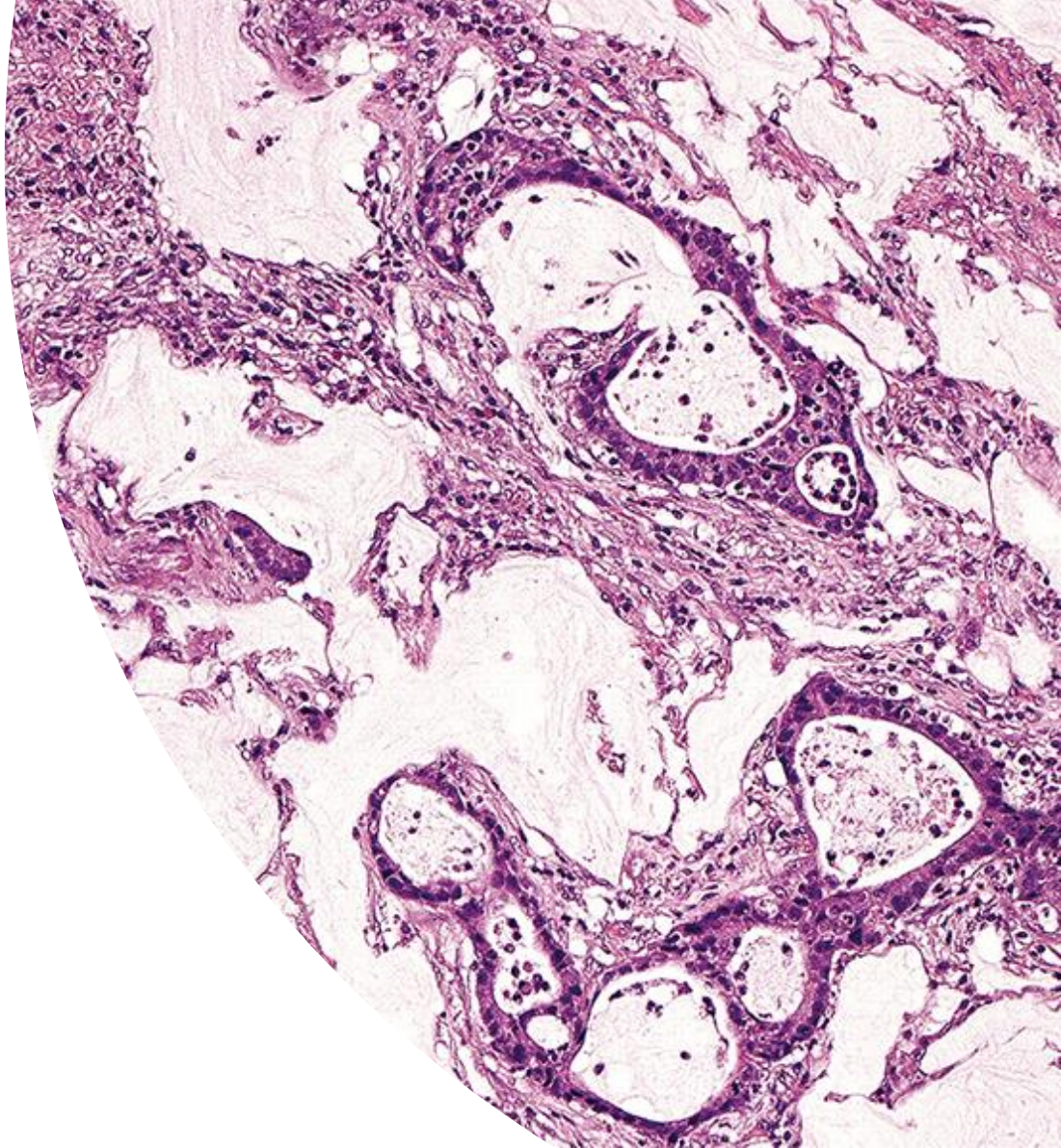
Invasive mucinous carcinoma

- **Goblet cell or columnar cell morphology**
- **Intracytoplasmic mucin.**
- **Often TTF1 negative**
- **CK7 positive, CK20, CDX2 focally positive**
- **Should be differentiated from pulmonary mets**
- **KRAS and NRG1 mutations common; EGFR rare**



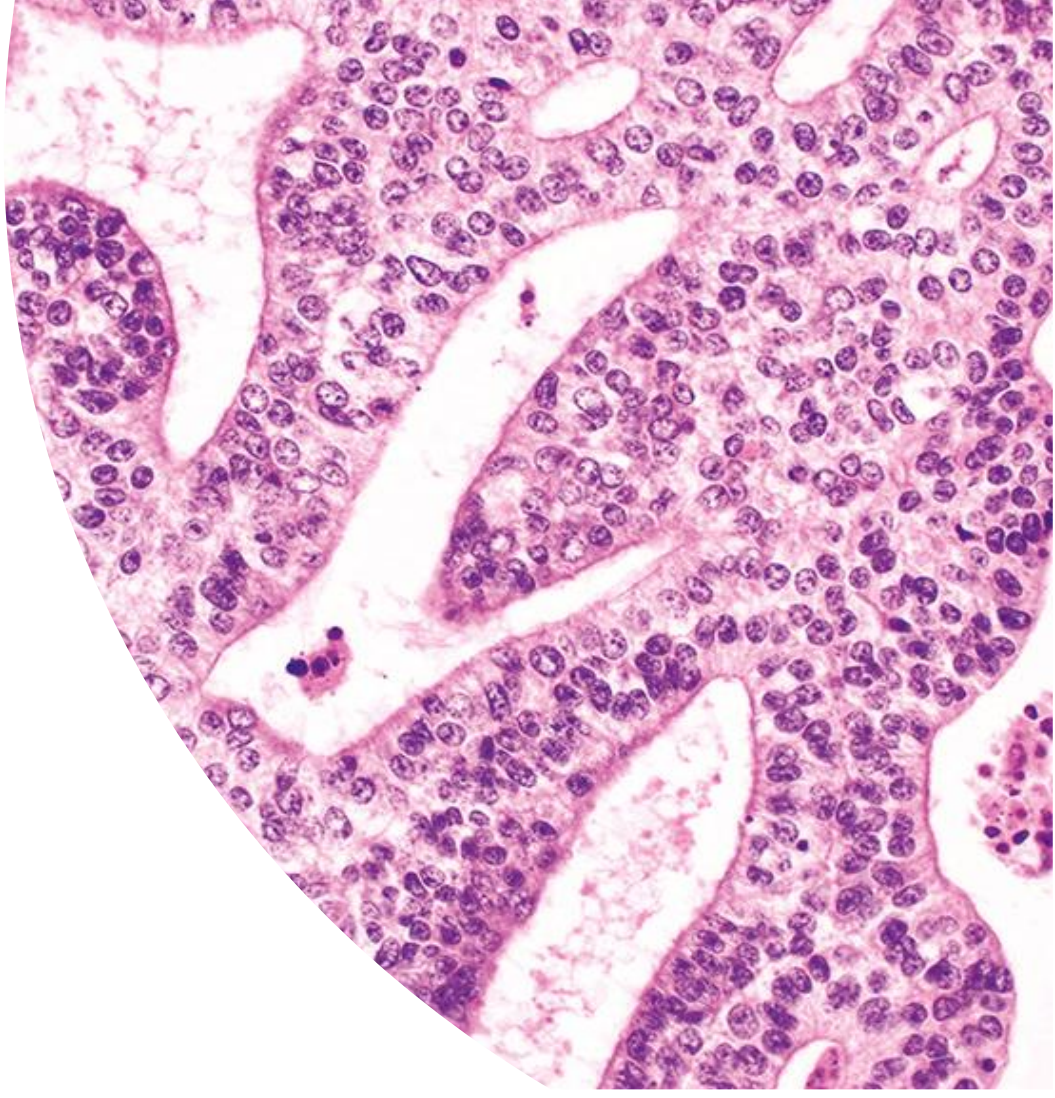
Colloid carcinoma

- **Extensive pools of extracellular mucin**
- **Distend alveolar spaces, destroy alveolar walls**
- **Often TTF1 negative**
- **CK7, CK20, CDX2 positive**
- **KRAS, STK11, PARP1 mutations common**
- **To be differentiated from GI mets**



Fetal adenocarcinoma

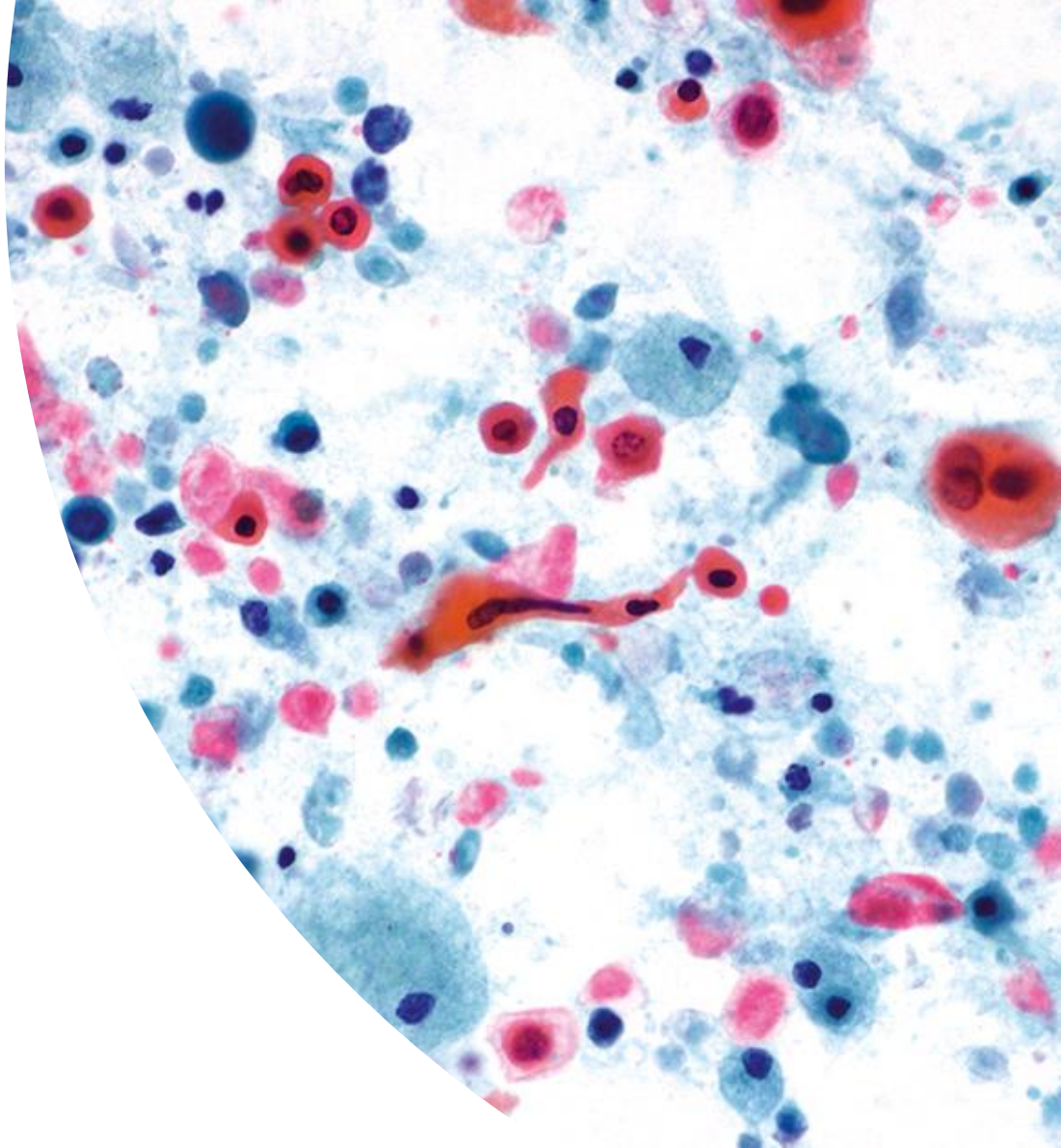
- **Resembles developing fetal lung in its pseudoglandular stage**
- **Low grade and high grade**
- **Abnormalities in β-catenin and aberrations in the WNT signalling pathway**
- **TTF1 positive in low grade**
- **Membranous β-catenin positive in high grade**
- **DICER1 and CTNNB1 mutations**



Squamous cell carcinoma

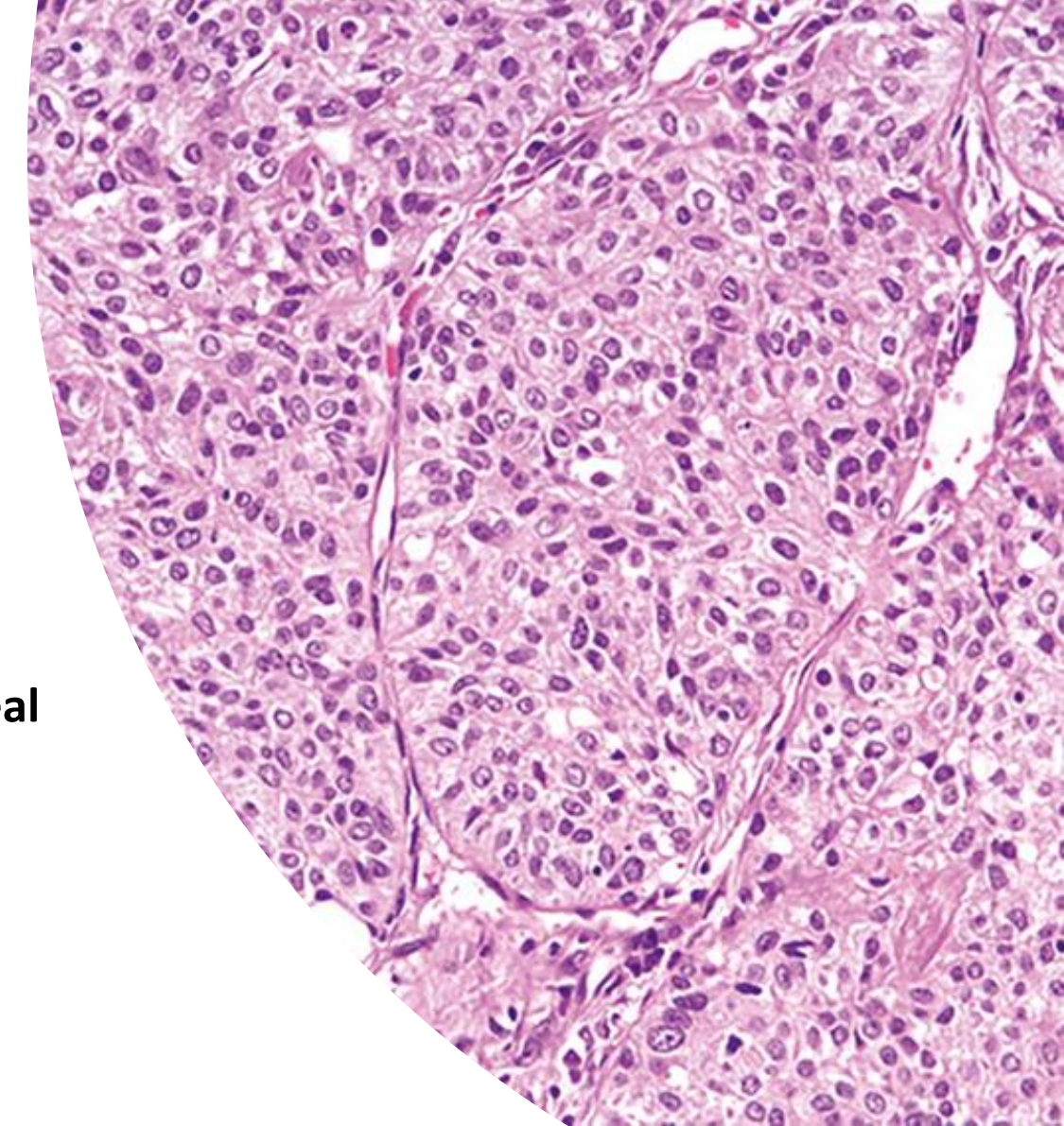
Squamous cell carcinoma

- Keratinization, intercellular bridges,
  - or IHC markers (p40,CK5/6, desmoglein, desmocollin, p63 positive, TTF1 negative)
- EGFR, ALK rearrangement may be positive



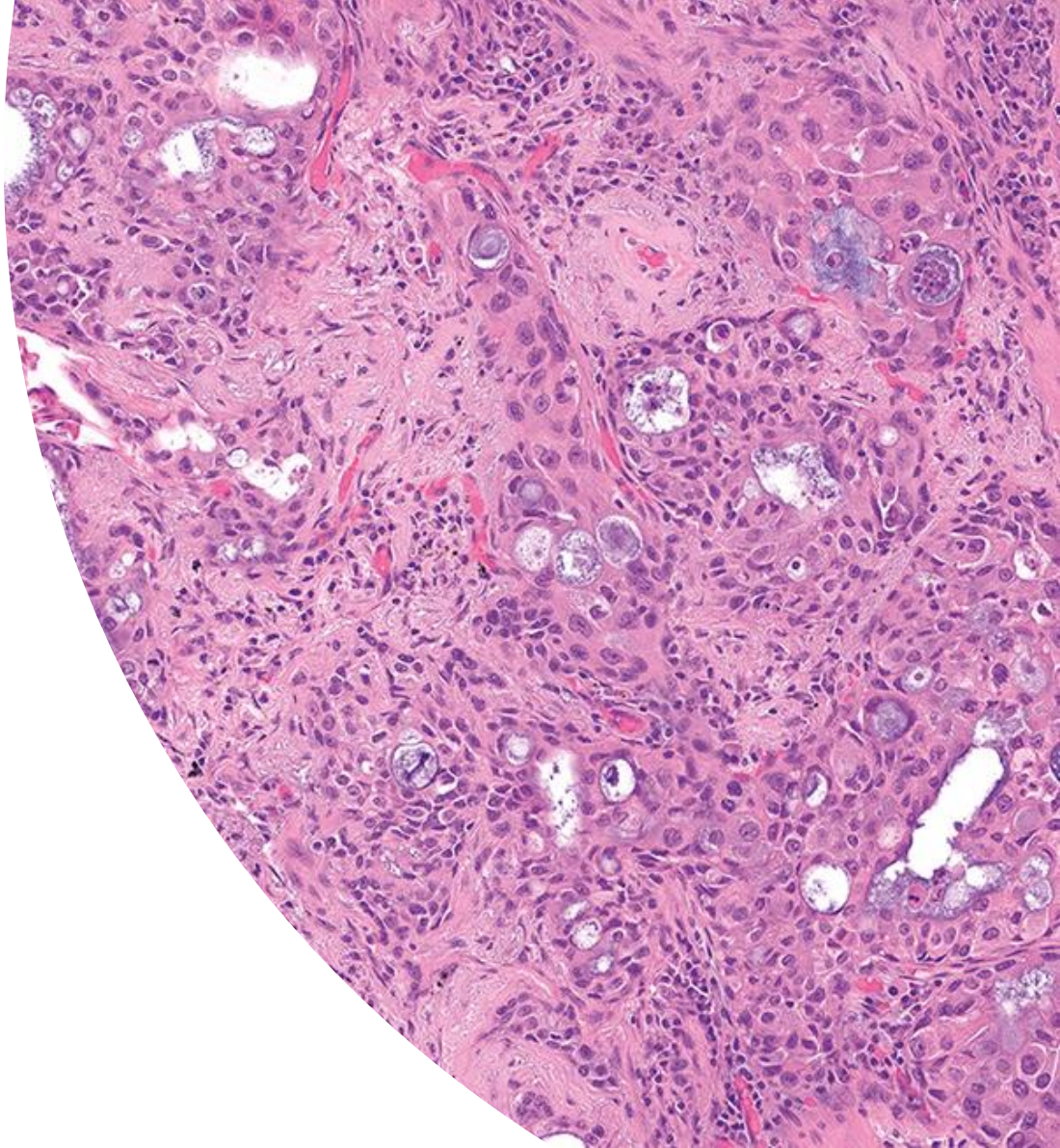
Lymphoepithelial carcinoma

- **Non-keratinizing SCC with**
  - **syncytial-appearing tumour cells,**
  - **vesicular nuclei,**
  - **distinct nucleoli**
  - **Lymphoplasmacytic infiltrate**
- **Exclusion of metastatic nasopharyngeal carcinoma clinically**
- **Often EBV positive**
- **TP53, KRAS, EGFR, ALK and ROS1 mutations mostly absent**
- **PD-L1 positive cases show good prognosis**



Adenosquamous carcinoma

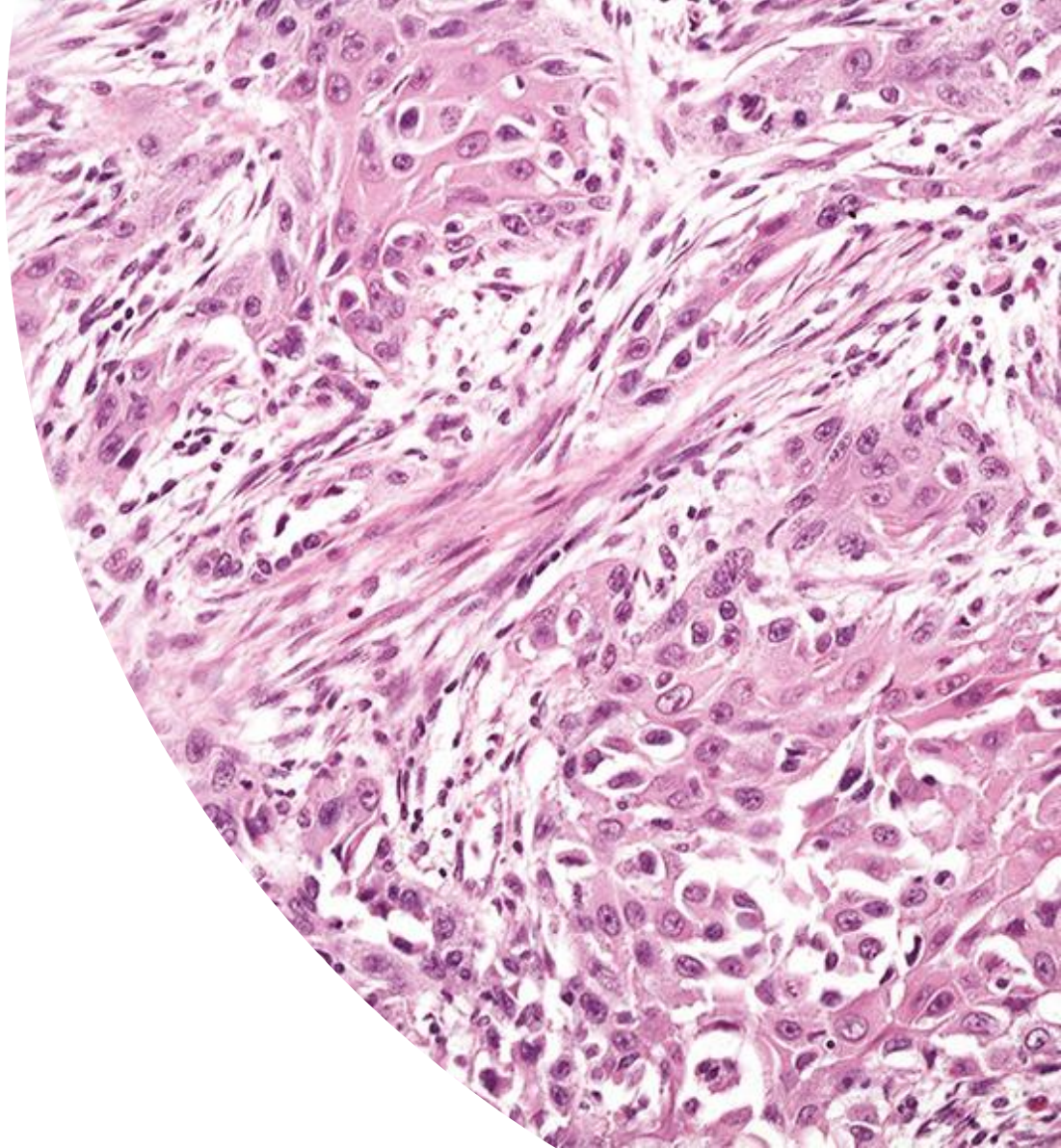
- **More than 10% of SCC and Adenocarcinoma component**
- **Common driver mutation**
- **EGFR, ALK, ROS1**



Sarcomatoid carcinomas

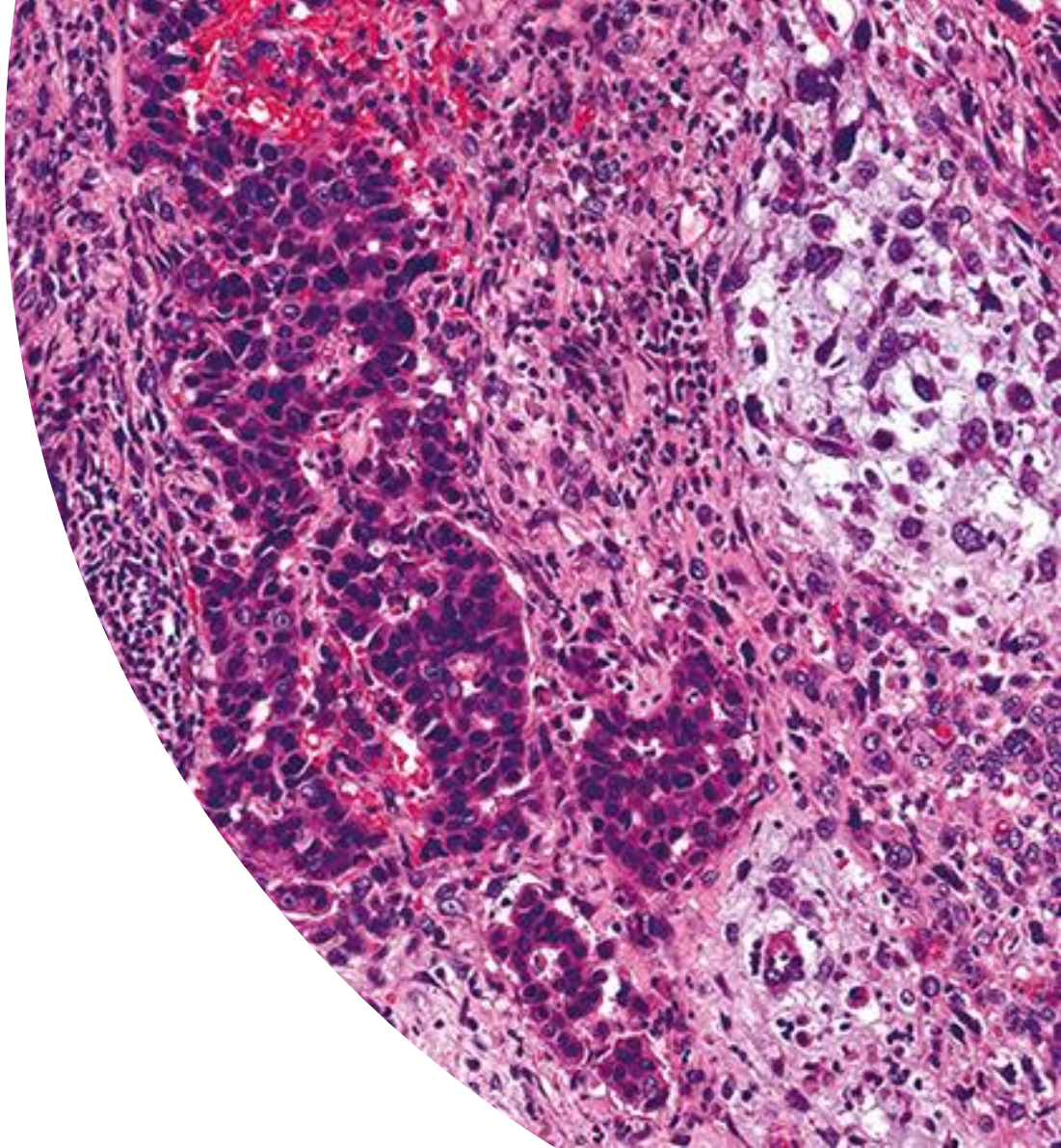
Pleomorphic carcinoma

- Poorly differentiated non-small cell lung carcinoma containing at least a 10% component of spindle and/or giant cells
- Diagnosis on resection
- EGFR, KRAS, MET and rarely ALK targetable mutations found in a subset



Carcinosarcoma

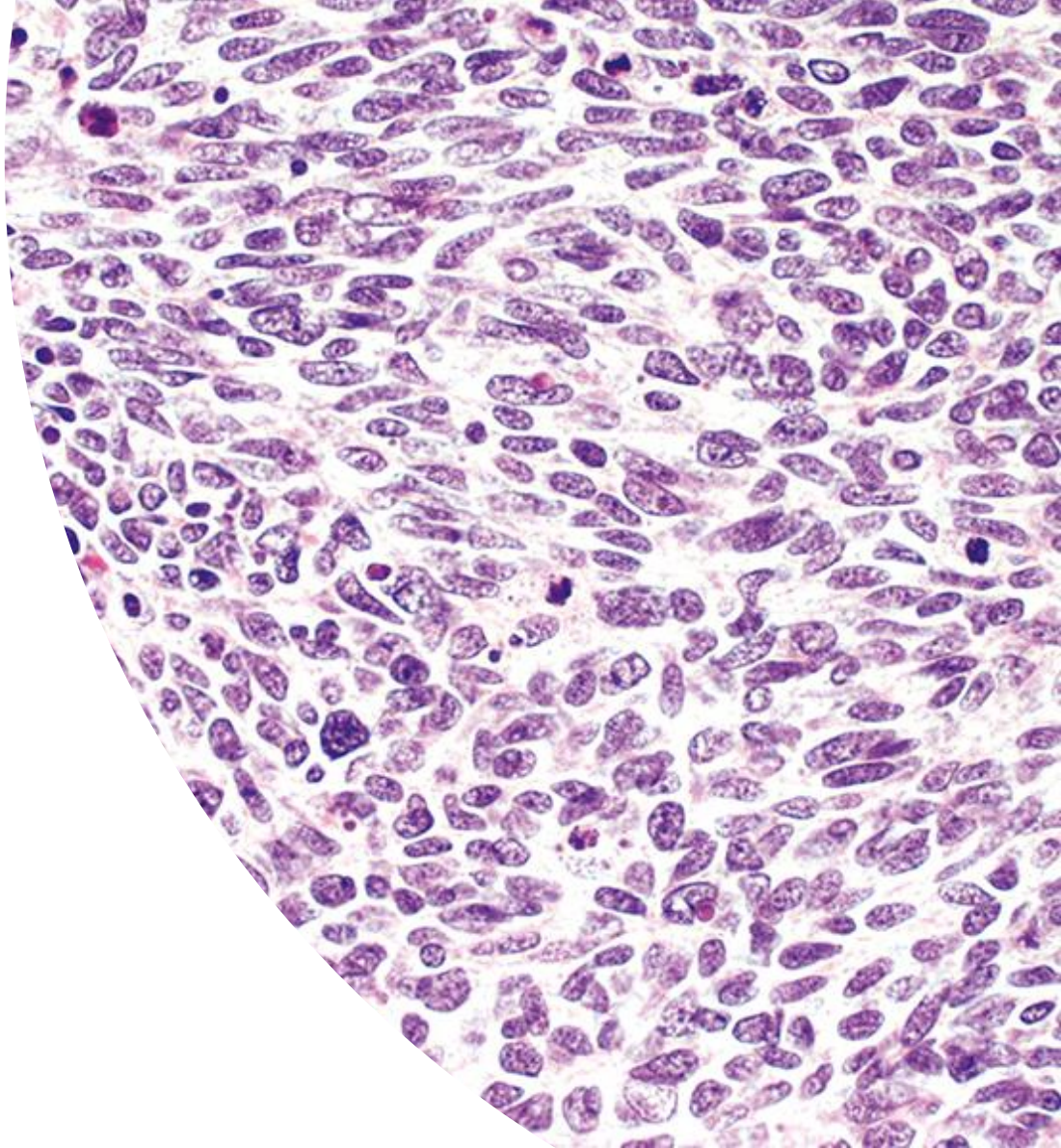
- Non-small cell lung carcinoma along with heterologous sarcomatous component e.g. rhabdomyosarcoma, chondrosarcoma
- May show mutations associated with carcinoma component



Neuroendorine carcinomas

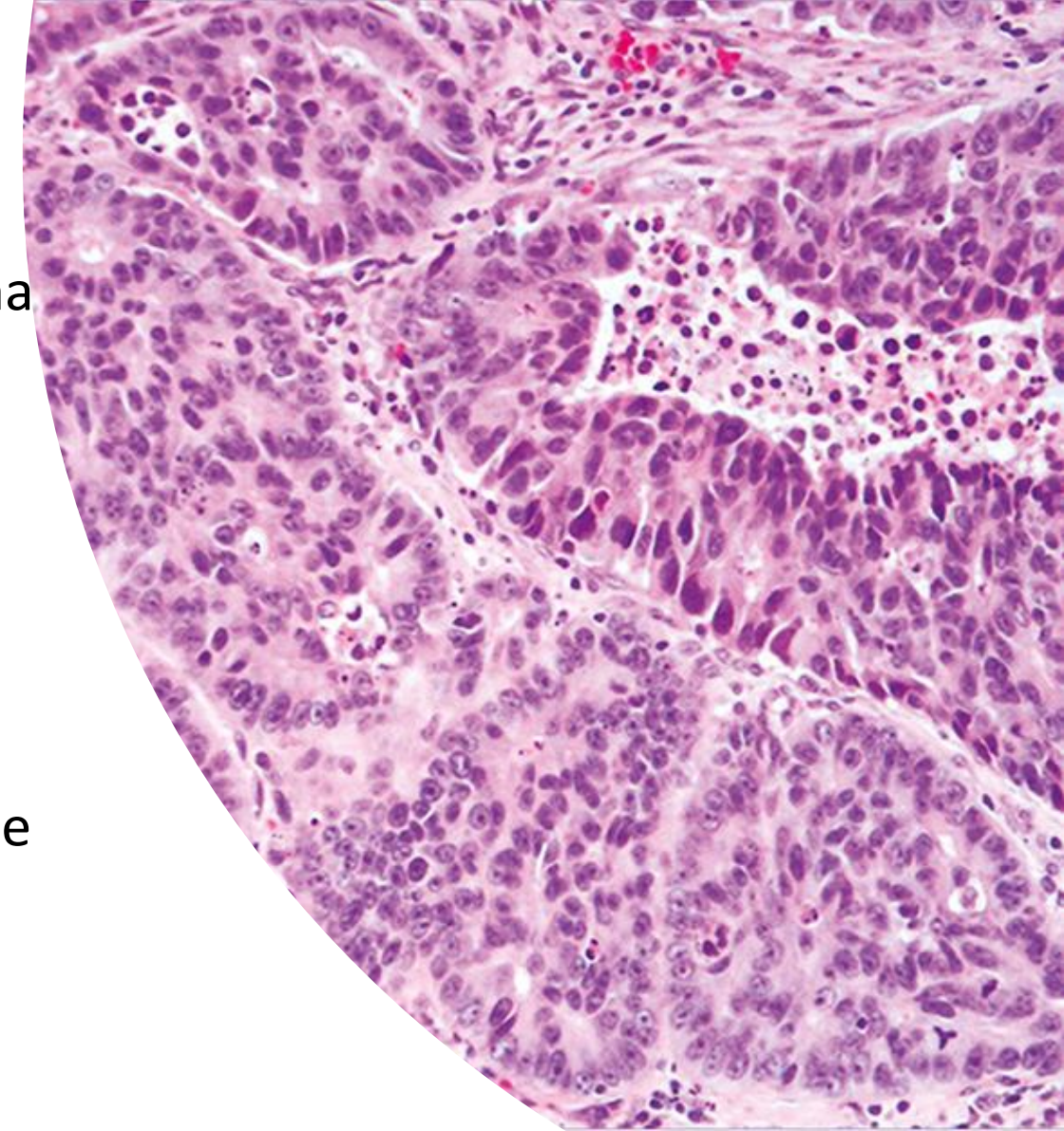
Small cell carcinoma

- Light microscopic diagnosis
- CK+, Synaptophysin+, Chromogranin +
- P53 and RB1 loss
- Mutation profiling not of benefit
- Modest response to immunotherapy + chemotherapy



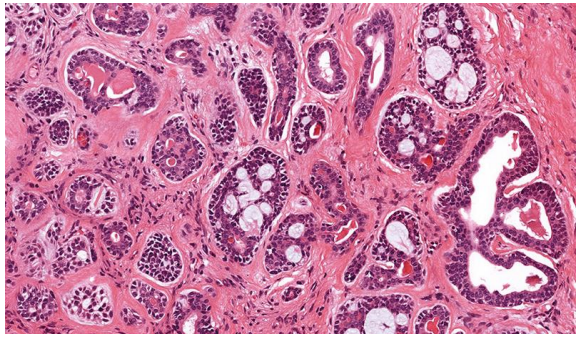
Large cell neuroendocrine carcinoma

- High-grade non-small cell carcinoma with neuroendocrine morphology
- Mitotic count> 10 mitoses/2 mm^2
- Expresses one or more neuroendocrine IHC markers.
- Combined LCNEC is an LCNEC with components of adenocarcinoma, squamous cell carcinoma, or spindle or giant cell carcinoma
- Low expression of EGFR and ALK, but such occurrence is seen

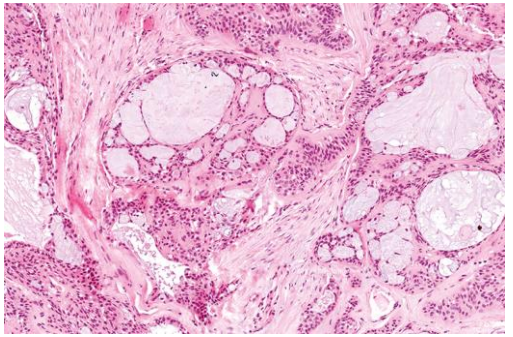


Salivary gland-like carcinomas

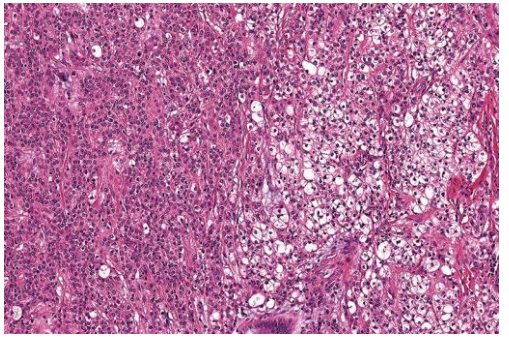
Salivary gland carcinomas



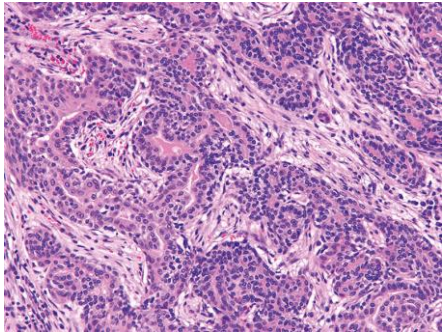
Adenoid cystic



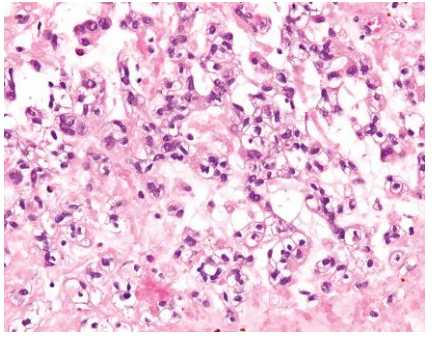
Mucoepidermoid



Hyalinizing clear cell



Epithelial-Myoepithelial

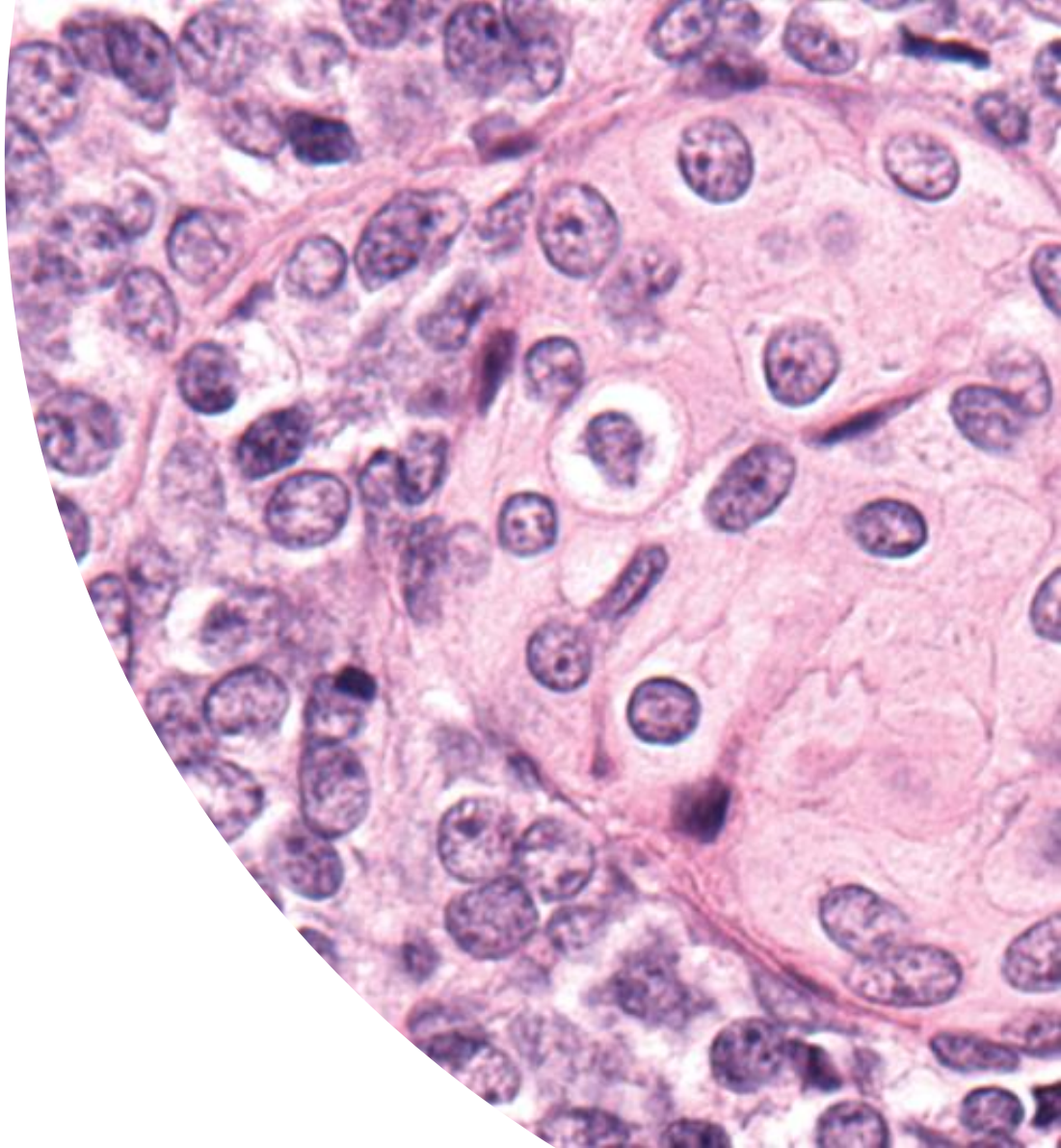


Myoepithelial

Others

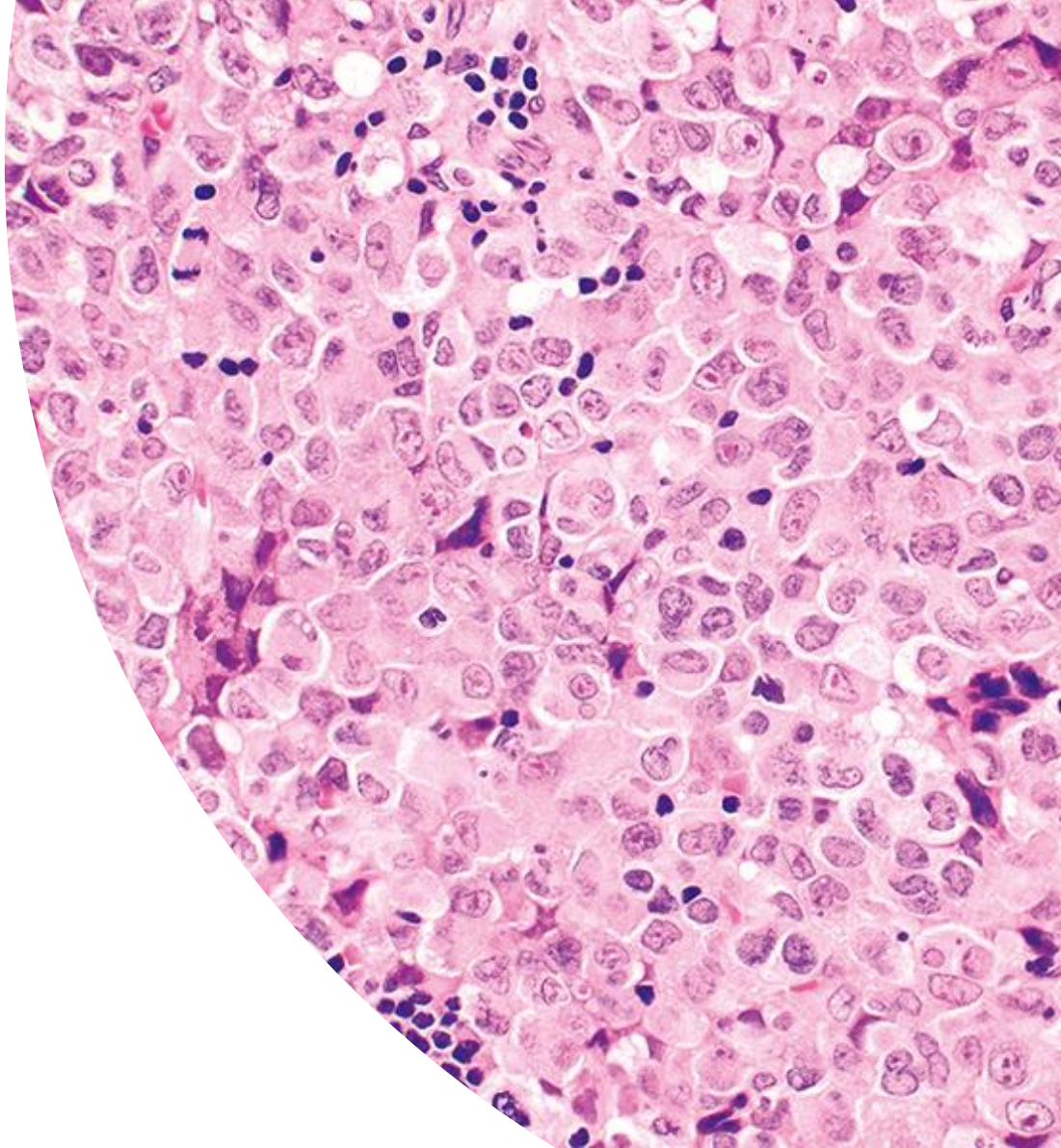
NUT Carcinoma

- Defined by presence of nuclear protein in testis (*NUTM1*) gene rearrangement
- Young patients
- Very aggressive
- Positive for NUT1 protein
- May show squamous differentiation



Thoracic SMARCA4-deficient undifferentiated tumour

- Diffuse sheets
- variably dyscohesive, large round to epithelioid cells
- Vesicular chromatin and prominent nucleoli
- Response to chemotherapy poor
- KRAS and STK11 mutations



Classification Having EGFR/ALK targetable mutations

- **Adenocarcinoma (non-mucinous)**
- **Squamous cell carcinoma**
- **Adenosquamous carcinoma**
- **Large cell carcinoma**
- **Sarcomatoid carcinoma**
- Neuroendocrine tumors
  - Small cell carcinoma
  - **Large cell neuroendocrine**
- Salivary gland type carcinoma
- Hematolymphoid tumors

Biomarkers

Diagnostic

- P40 for SCC, TTF1 for adenocarcinoma
- Recent: circulating tumor DNA, circulating tumor cells : Not validated

Predictive

- Mutational profile: **EGFR** (including T790M), **ALK, ROS1** in NSCLC
  - Potentially important: MET 14 exon skipping mutations, RET rearrangements, BRAFV600E, KRAS p.612C
- Predictor for immunotherapy: **PD-L1**
  - Different IHC tests for predicting response to different drugs
  - Potential alternatives: Tumor mutational burden, Microsatellite instability

Mutational profile Biomarkers

Type of specimen

- Any (FNA material, from slide, needle core biopsy blocks, biopsy/FNA from secondaries, pleural fluid, circulating tumor DNA)

How?

- EGFR, KRAS, BRAF (indel mutations): PCR/qPCR, NGS on extracted DNA
- ALK, ROS1 (translocations): FISH/CISH, IHC for mutant protein On tissue blocks/Cytology slides,
  - NGS On extracted DNA/RNA
- MET 14 exon skipping mutations, RET rearrangements: NGS, especially RNA-Seq

Why?

- To find targetable genes
- In case of high survival (minimally invasive carcinoma, ACC etc) not needed
- In case of histology unlikely to reveal any mutations, probably not needed

Molecular testing: Basic principles

Protein overexpression (e.g. PD-L1): IHC

Indel mutations with known sequence (EGFR, KRAS,BRAF) : PCR/qPCR

Amplification (ERBB2): In Situ Hybridization

Translocations(ALK, ROS1, RET rearrangements): In Situ Hybridization

All mutations: NGS

- indel mutations(by DNA-seq)
- amplifications (by both DNA-seq and RNA seq)
- translocations (by both DNA seq and **RNA seq**)