# Histopathological diagnosis of CUP

Dr Karin Oien

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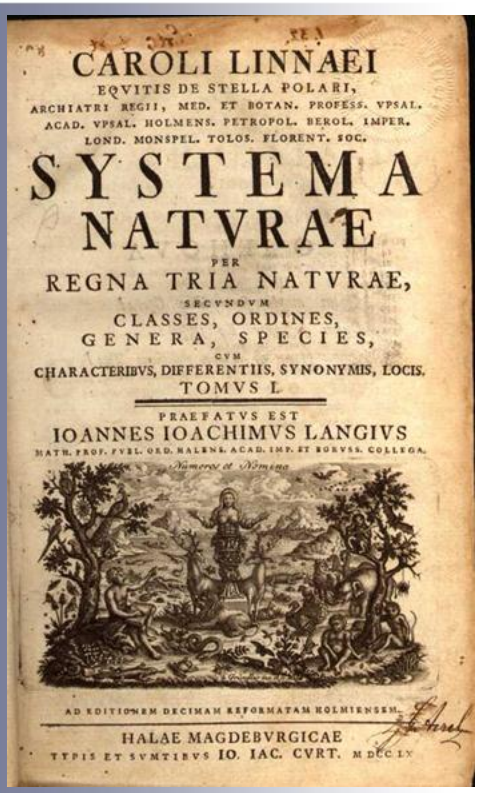
# Disclosure slide

- Dr Karin Oien has no financial interests in any company mentioned in this presentation.
- Dr Karin Oien is conducting research in collaboration with BioTheranostics and with the research group of Professor David Bowtell; their contribution to the study is intellectual and to provide molecular analyses but not otherwise financial.

# Outline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HALAE MAGDEBVRGICAE
TYPIS ET SVMTIBVS IO. IAC. CVRT. MDCCLX

- Pathological approach to cancer classification in general; and thus…
- Pathological approach to CUP incl. IHC
  - Developments
- Molecular pathology for CUP

sourced from http://bibbild.abo.fi/Linneana/ category:books

# Aims of pathology and cancer classification

Pathology aims:

1. Identification of disease
2. Diagnosis
3. Prognosis: prediction of outcome
4. Prediction of treatment benefit

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2. What does tumour look like?
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Cancer classification based on:

1. Where is the tumour?
2. What does tumour look like?
3. Now, what is its molecular profile?

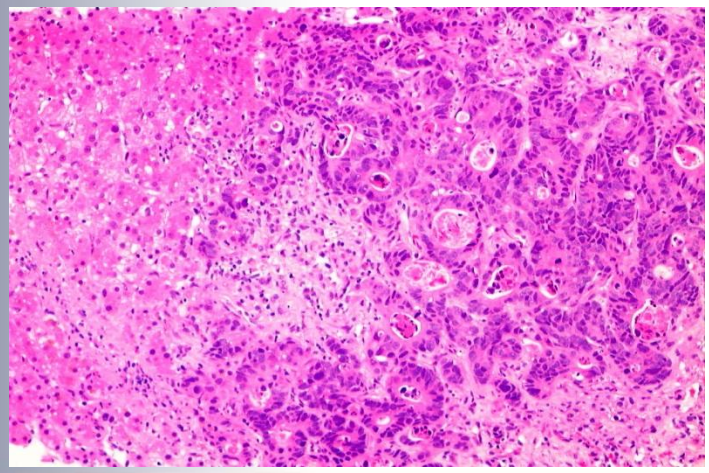
Establishing cancer classification i.e. diagnosis, prognosis & prediction, is therefore a main aim of cancer pathology.

# Current classification by cancer type & site

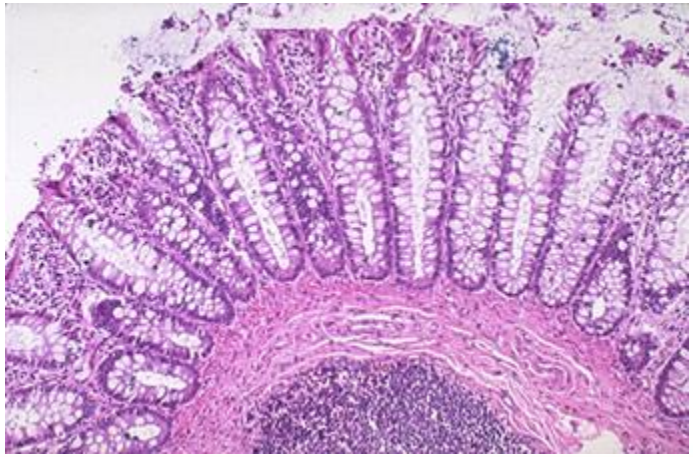
- Based on resemblance to normal tissue counterpart by morphology

**Normal liver this end**

**Abnormality this end: metastatic colon cancer**



**Normal colon lining for comparison**



# Current classification by cancer type & site

- Based on resemblance to normal tissue counterpart by morphology
- Classification by type & site is current initial predictor of outcome/treatment response

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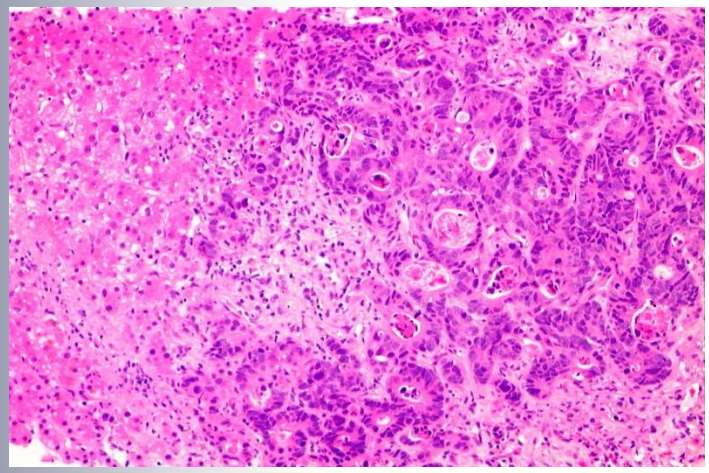

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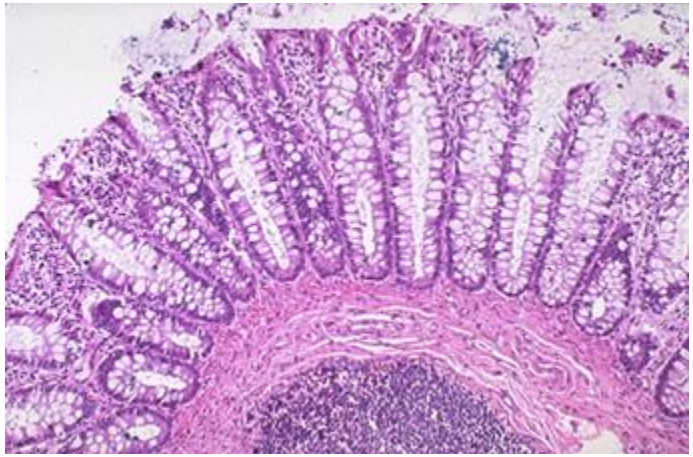
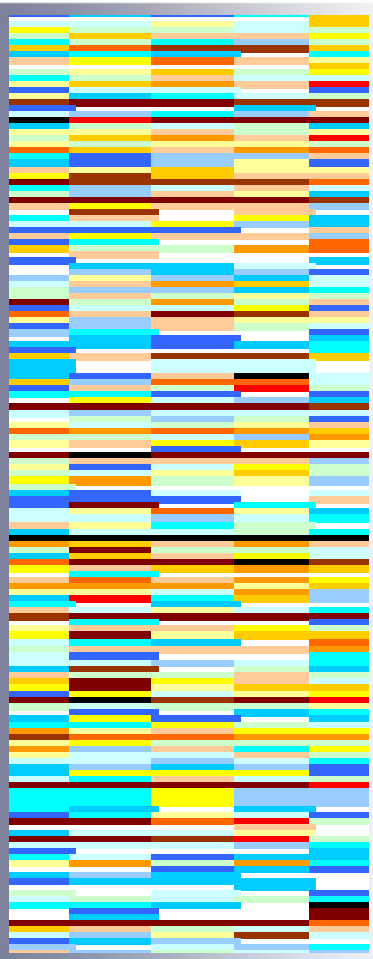
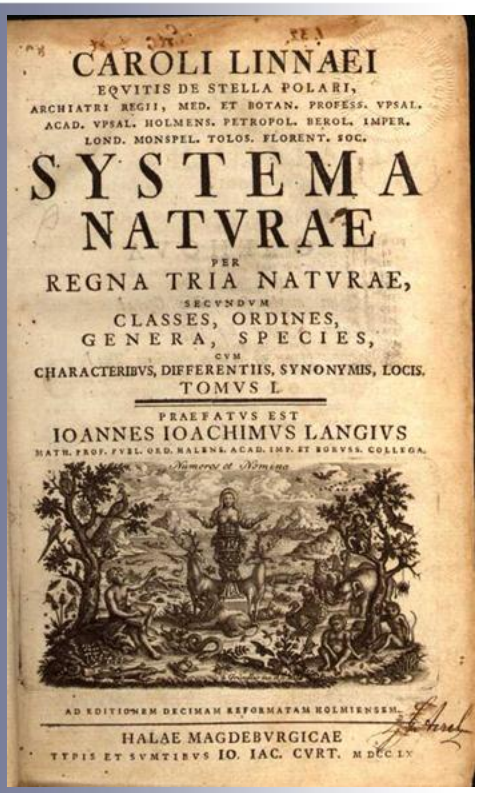


# Tissue-specific genes as cancer biomarkers for cancer (tissue) type and site



- Tumours are derived from specific tissues
- Normal tissue-specific morphology and gene expression is partly retained in cancers, both primary and metastatic
- These tissue-specific markers can be used for cancer classification
  - Immunohistochemistry (IHC) for protein (standard in pathology)
  - Molecular profiling for RNA
- Used in panels of multiple markers

# Outline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- Pathological approach to cancer classification in general; and thus…
- **Pathological approach to CUP incl. IHC**
  - Developments
- Molecular pathology for CUP

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# CUP: clinical and pathological problem

- CUP occurs mainly in common sites of metastasis:
  - Solid organs: liver, lung, bone, brain
  - Lymph nodes
  - Serous cavities
- But “uncertain” tumours, metastatic or primary, may occur anywhere
  - Clinical & pathological work-up similar
- Aim of pathology in CUP is optimal tumour classification to enable optimal patient management…

Image from **Royal College of Pathologists**

# Classification of cancer including CUP: A stepwise pathological approach..plus IHC...

**Step 1: identify broad cancer type**

- Carcinoma
- Melanoma
- Lymphoma/ leukaemia
- Sarcoma
- (Neuro-glial tumours)

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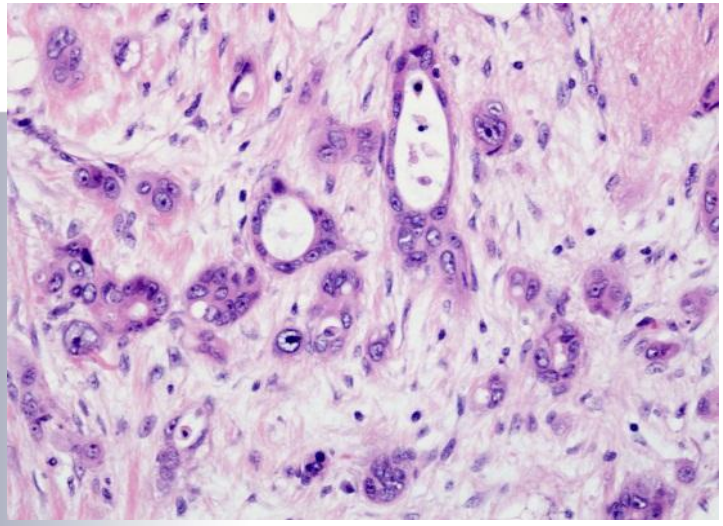
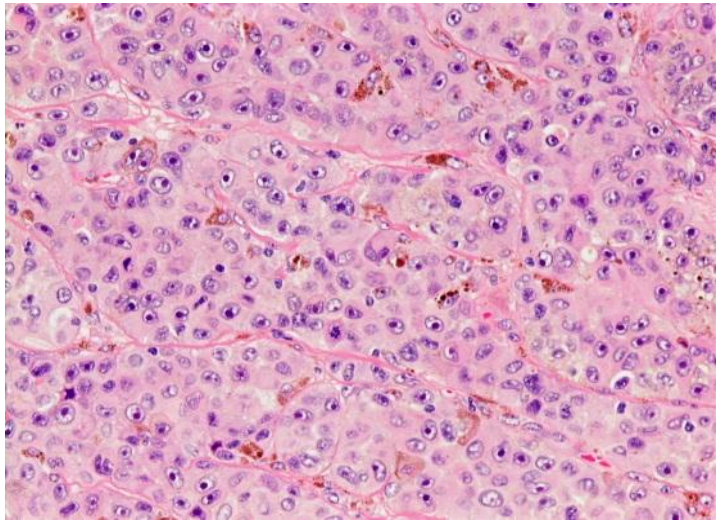
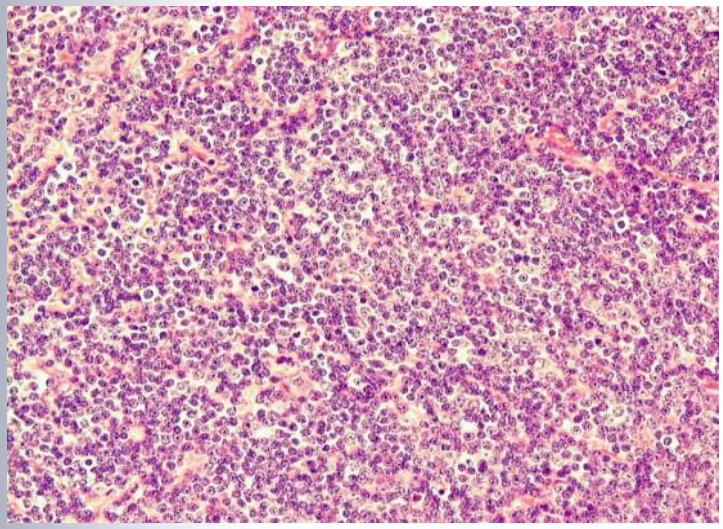
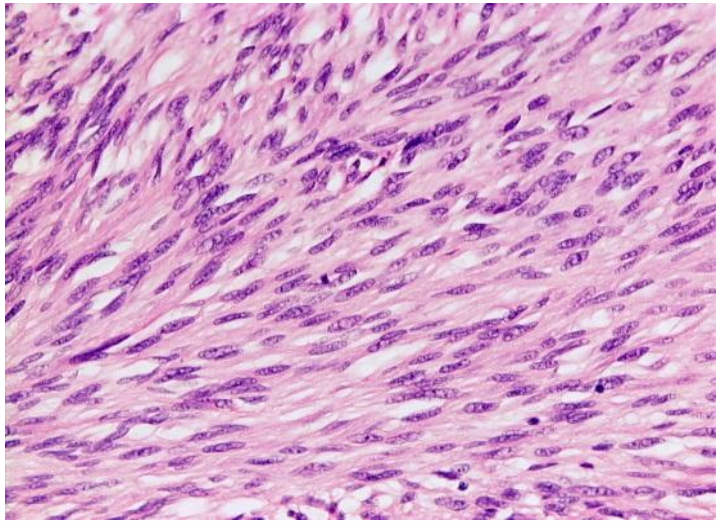
# Step 1: Identify broad cancer type









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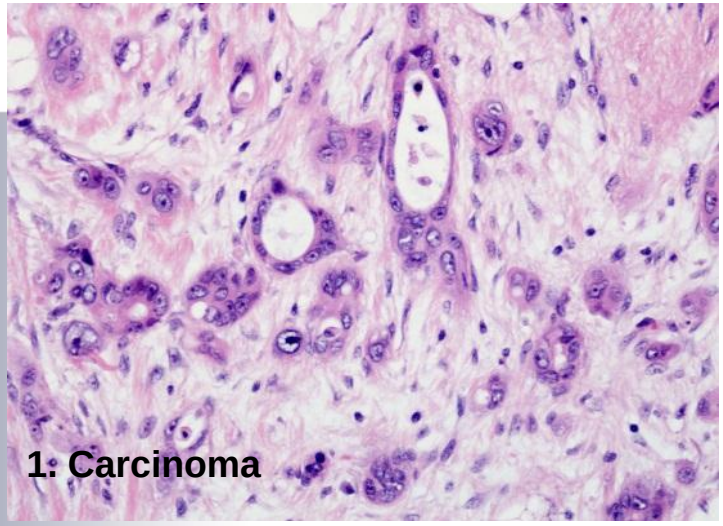
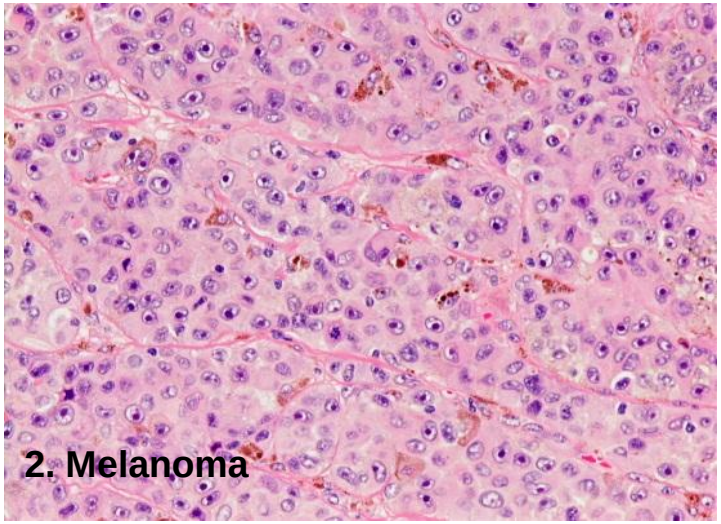
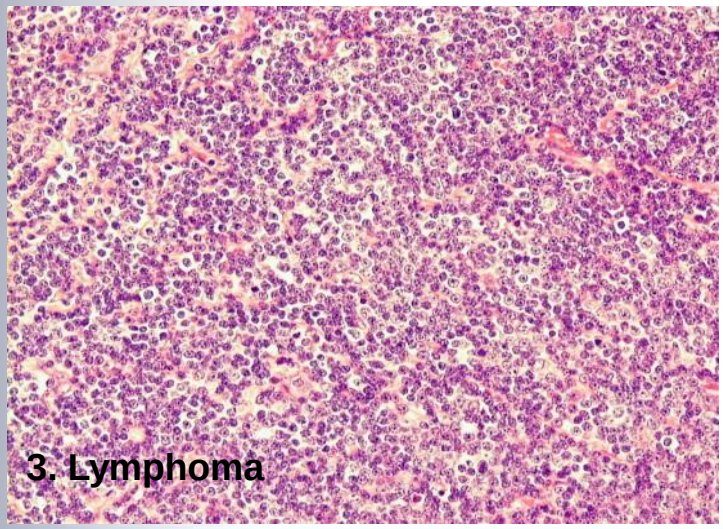
# Step 1: Identify broad cancer type



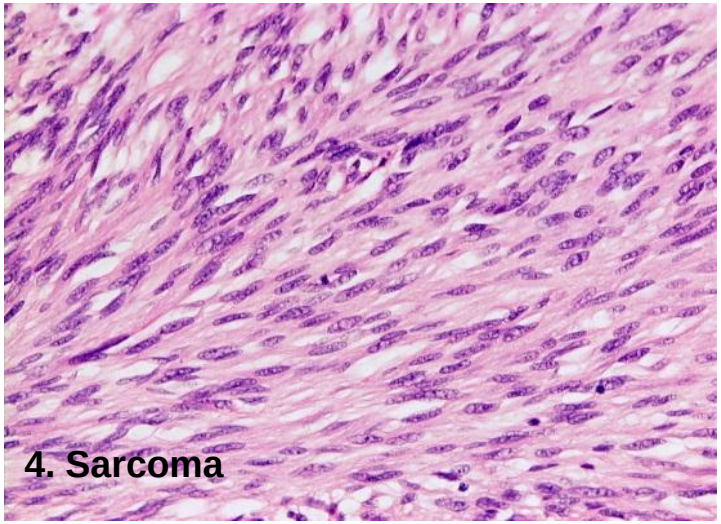
1. Carcinoma



2. Melanoma



3. Lymphoma



4. Sarcoma

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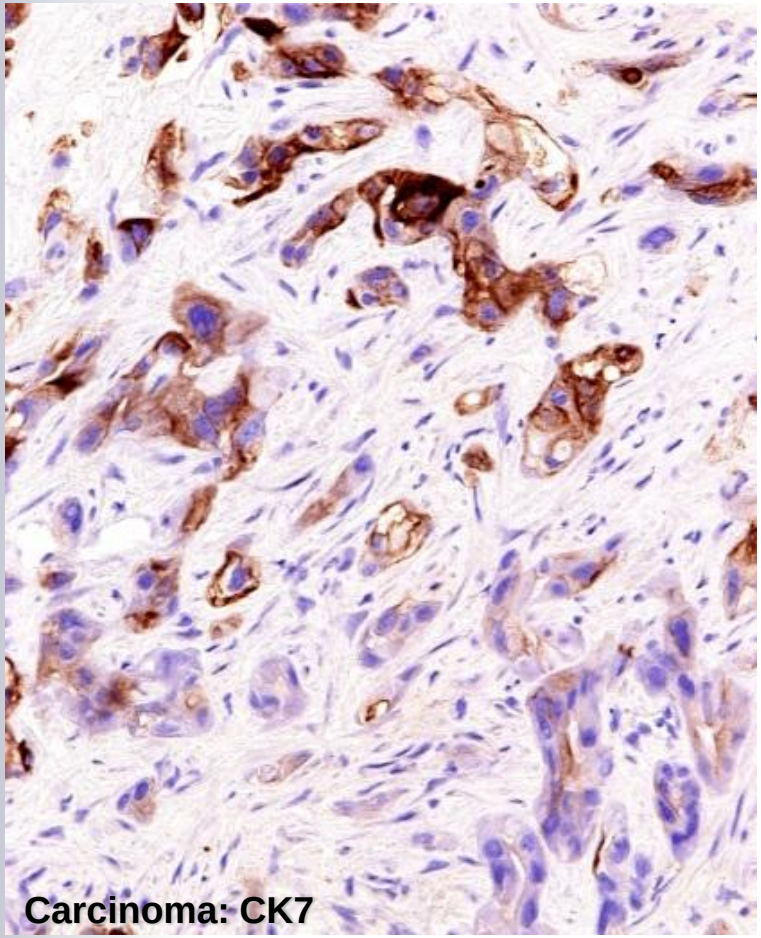
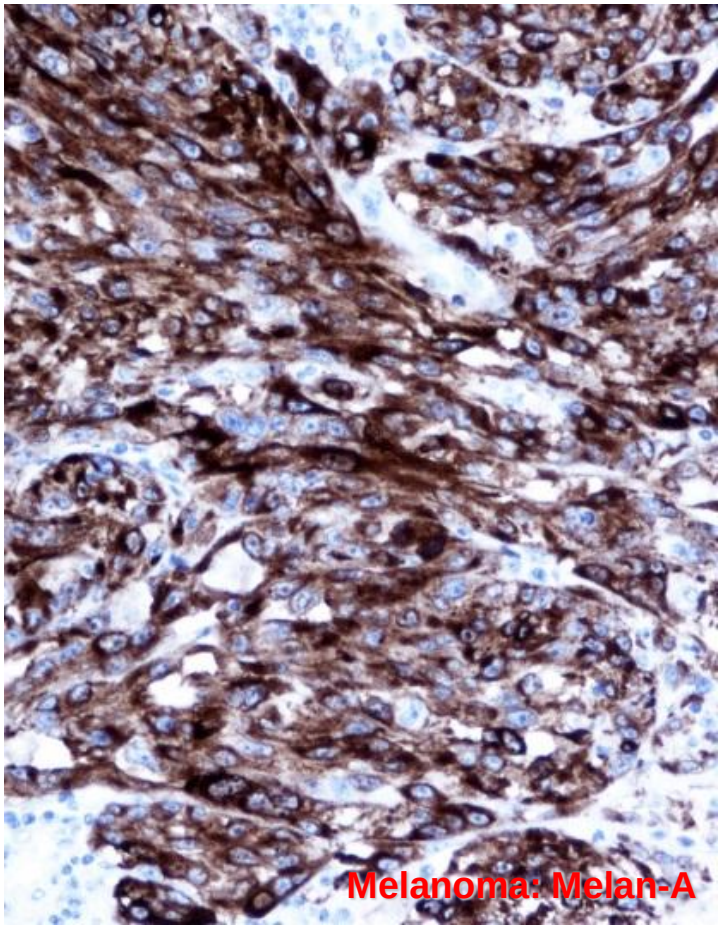
# Immunohistochemistry for CUP: Step 1: Identify broad cancer type

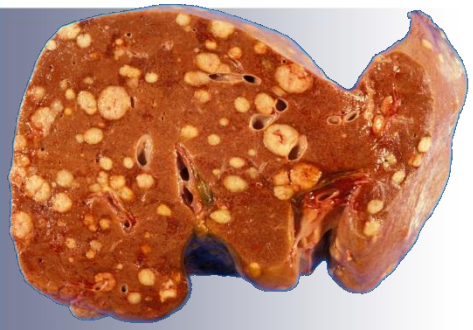
| | |
|---|---|
| Carcinoma | Cytokeratins and other epithelial markers e.g. **AE1/3**, CK7, CK20, CK5, EMA |
| Melanoma | **S100**, Melan-A, HMB45 |
| Lymphoma/ leukaemia | **CLA**, CD20, CD3, CD138, CD30 etc. |
| Sarcoma | Vimentin, actin, desmin, S100, c-kit etc |

# Immunohistochemistry for CUP: Step 1: Identify broad cancer type



# CUP: Step 1: Broad cancer type, comment



- CUP equates to carcinoma
- Other broad tumour types (lymphoma, melanoma, sarcoma etc) excluded from CUP by definition but in practice need considered and excluded during work-up of uncertain tumours, metastatic or primary

Oien 2009 Semin Oncol.

# Classification of cancer including CUP: A stepwise pathological approach..plus IHC...

**Step 1: identify broad cancer type**

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**Step 2: if carcinoma or related, identify subtype**

- **Adenocarcinoma**
- **Squamous ca.**
  - Transitional ca.
- **Solid organ ca.** (hepatocellular, renal, thyroid, adrenal)
- Neuroendocrine ca.
- (Germ cell tumour)
- (Mesothelioma)

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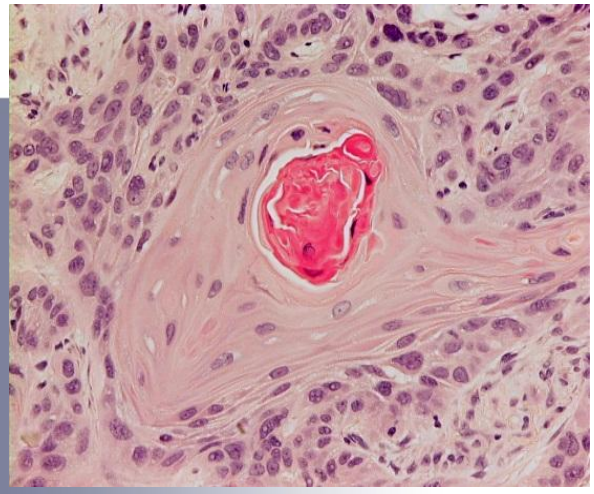
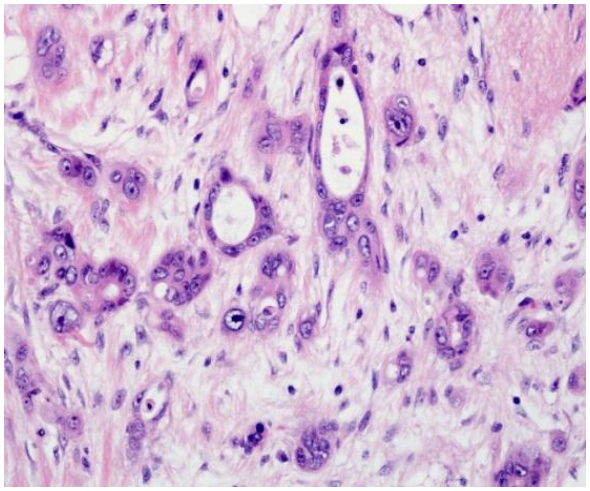
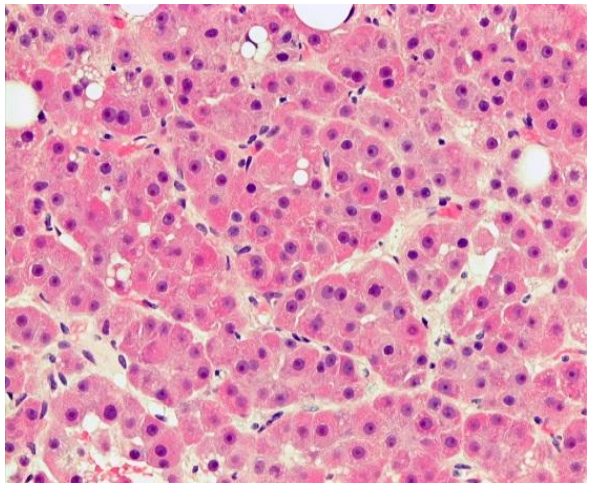
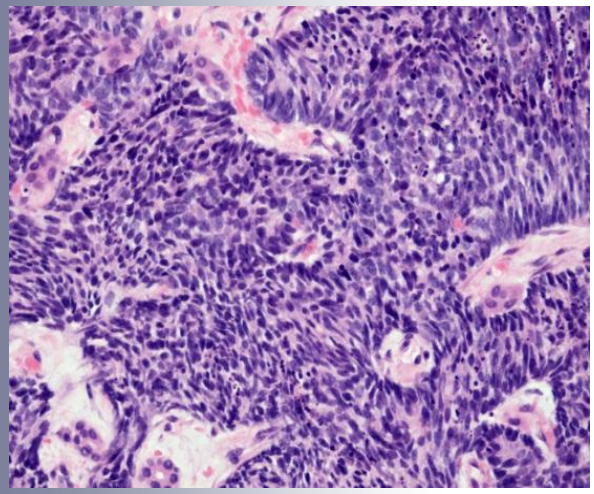
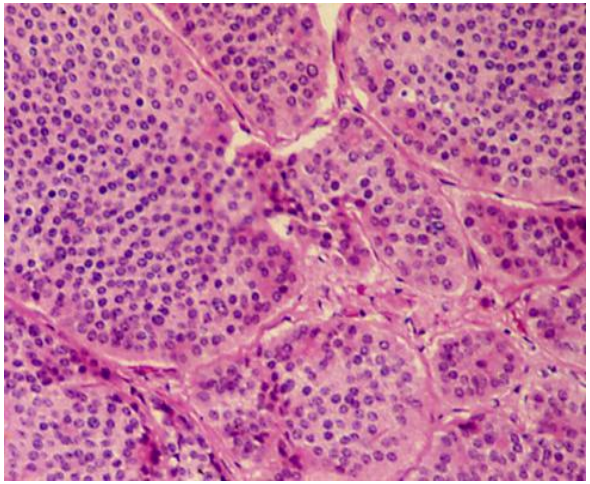
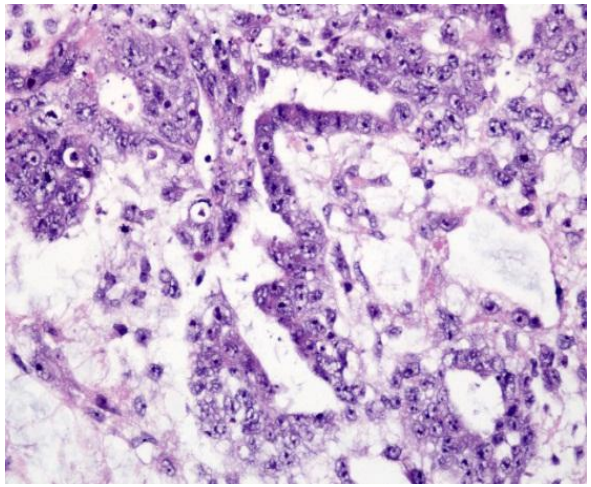
# CUP Step 2: Identify subtype of carcinoma

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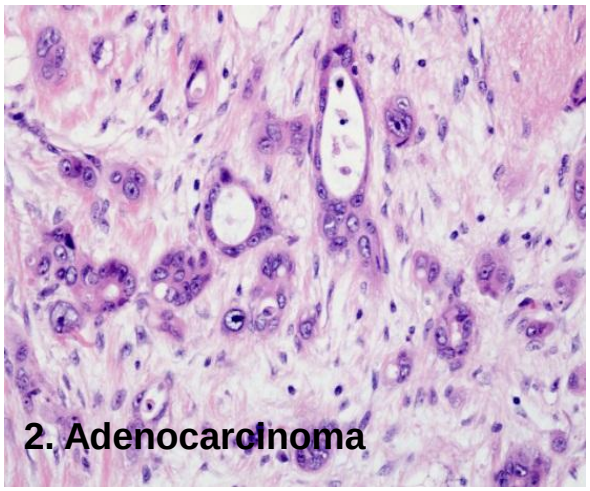
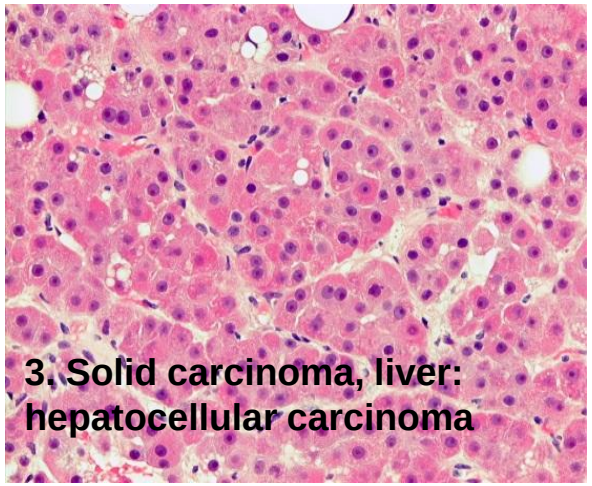
# CUP Step 2: Identify subtype of carcinoma

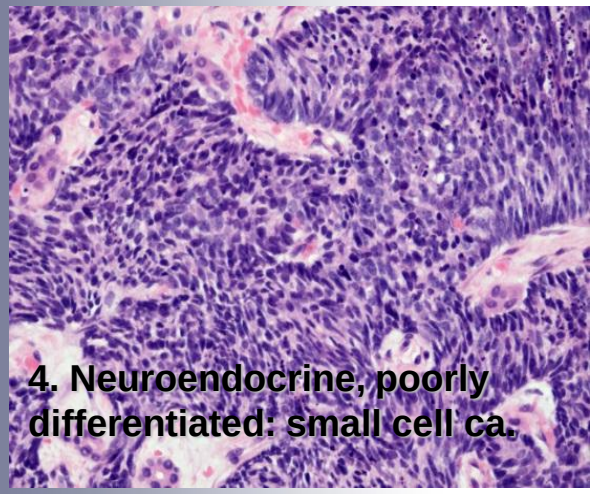
1. Squamous carcinoma



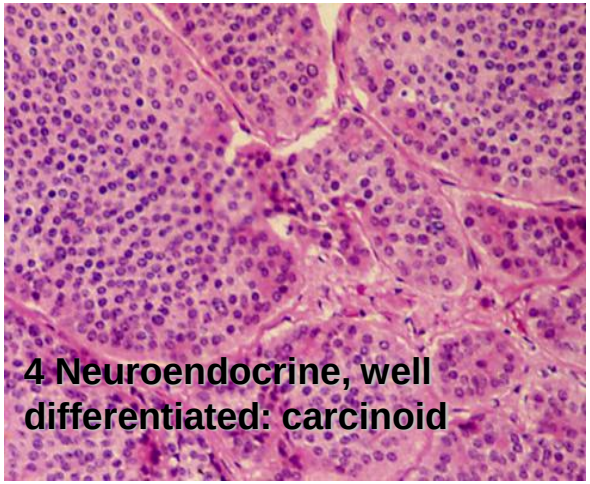
2. Adenocarcinoma



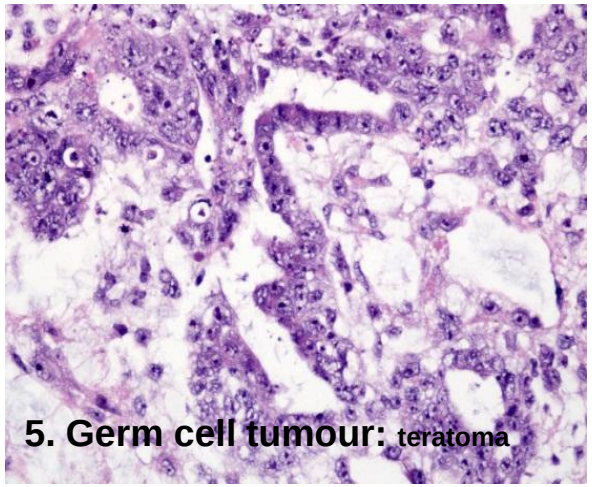
3. Solid carcinoma, liver: hepatocellular carcinoma



4. Neuroendocrine, poorly differentiated: small cell ca.



4 Neuroendocrine, well differentiated: carcinoid



5. Germ cell tumour: teratoma

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# Immunohistochemistry for CUP: Step 2: Identify subtype of carcinoma

| | |
|---|---|
| **Adenocarcinoma** | **CK7, CK20, PSA** and other adenoca markers |
| **Squamous ca** | **CK5, p63** |
| **Transitional ca** | CK7, CK20, urothelin |
| **Neuroendocrine ca** | **Chromogranin, CD56**, synaptophysin, TTF1 |
| **Solid ca**: renal | **RCC**, CD10, PAX8, Napsin A |
| **Solid ca**: liver | **Hepar1**, CD10, glypican-3 |
| **Solid ca**: thyroid | **TTF1**, thyroglobulin, PAX8 |
| Solid ca: adrenal | **Melan-A**, inhibin |
| (Germ cell tumour) | **OCT4, PLAP**, HCG, AFP |
| (Mesothelioma) | Calretinin, mesothelin, WT1, D2-40 |

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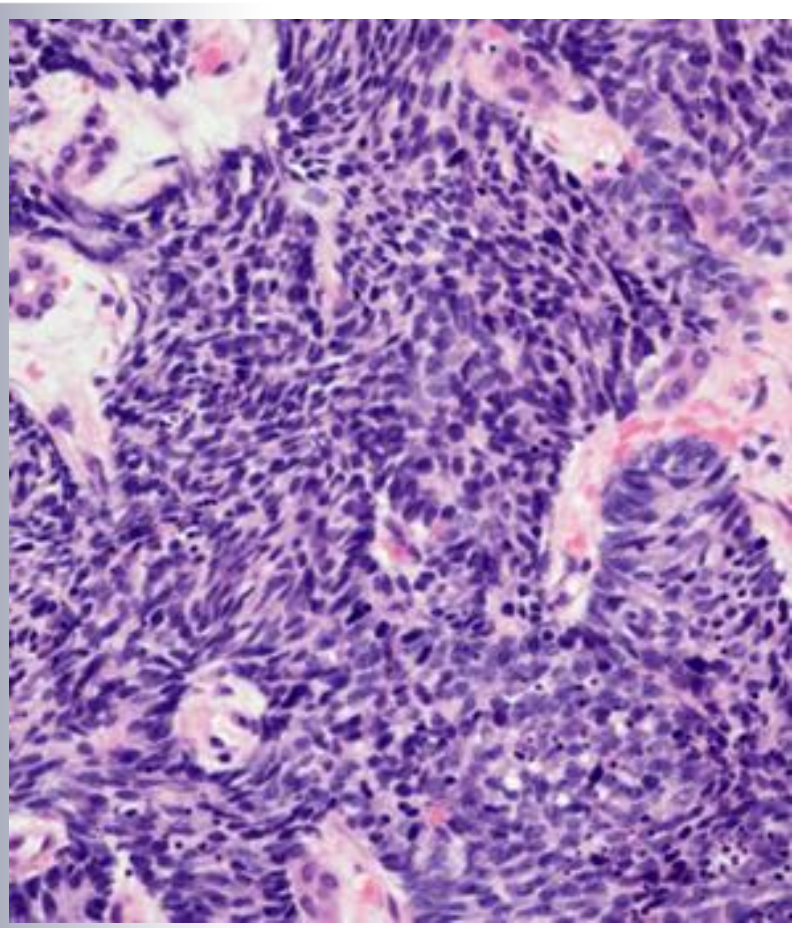
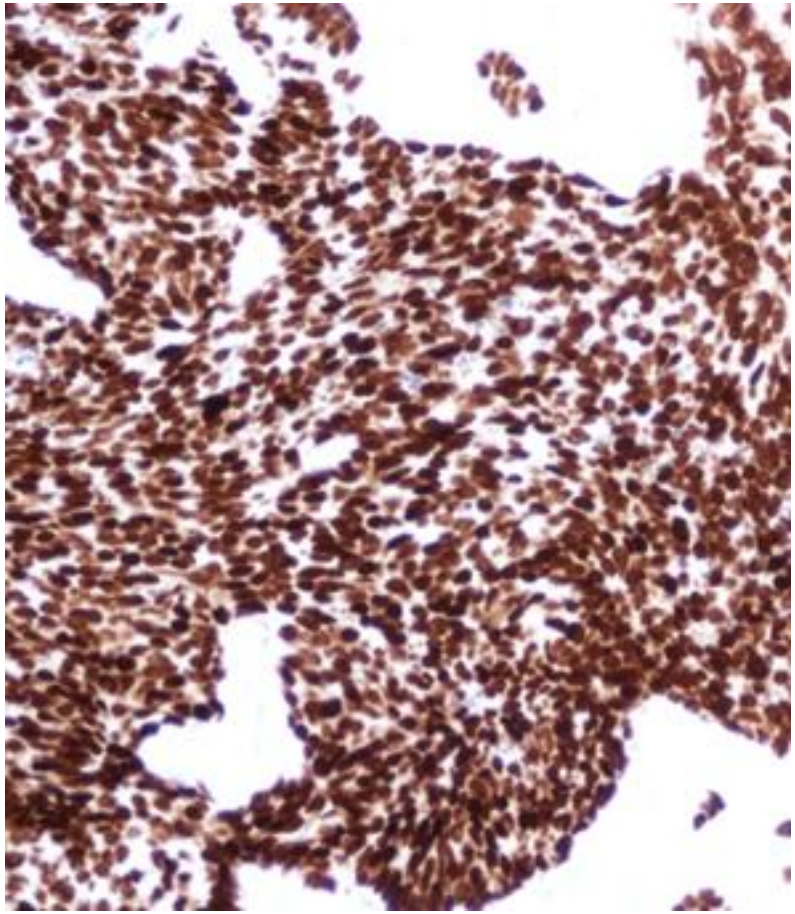
# Immunohistochemistry for CUP: Step 2: If carcinoma, identify subtype





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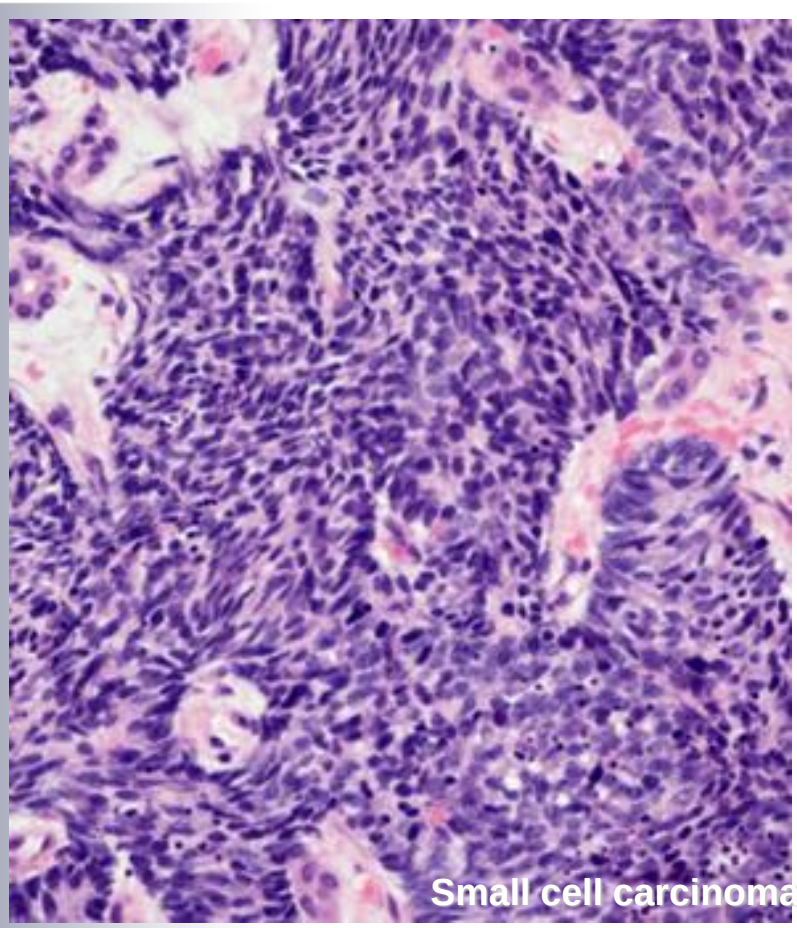
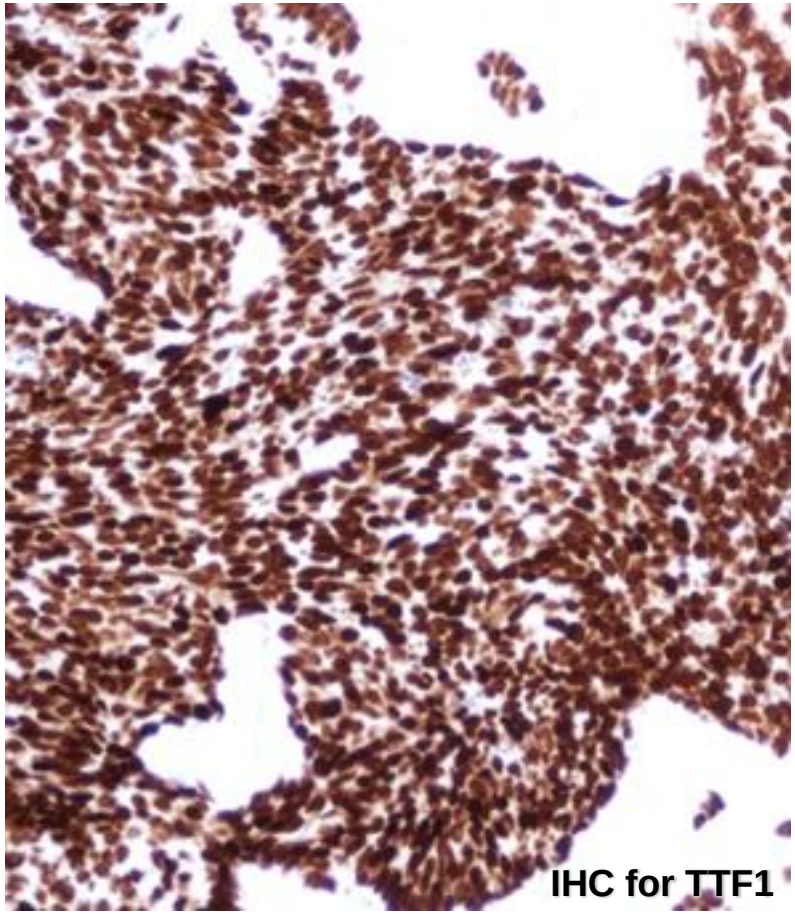


Small cell carcinoma



IHC for TTF1

# Common carcinoma subtypes in CUP

**Step 1: identify broad cancer type**

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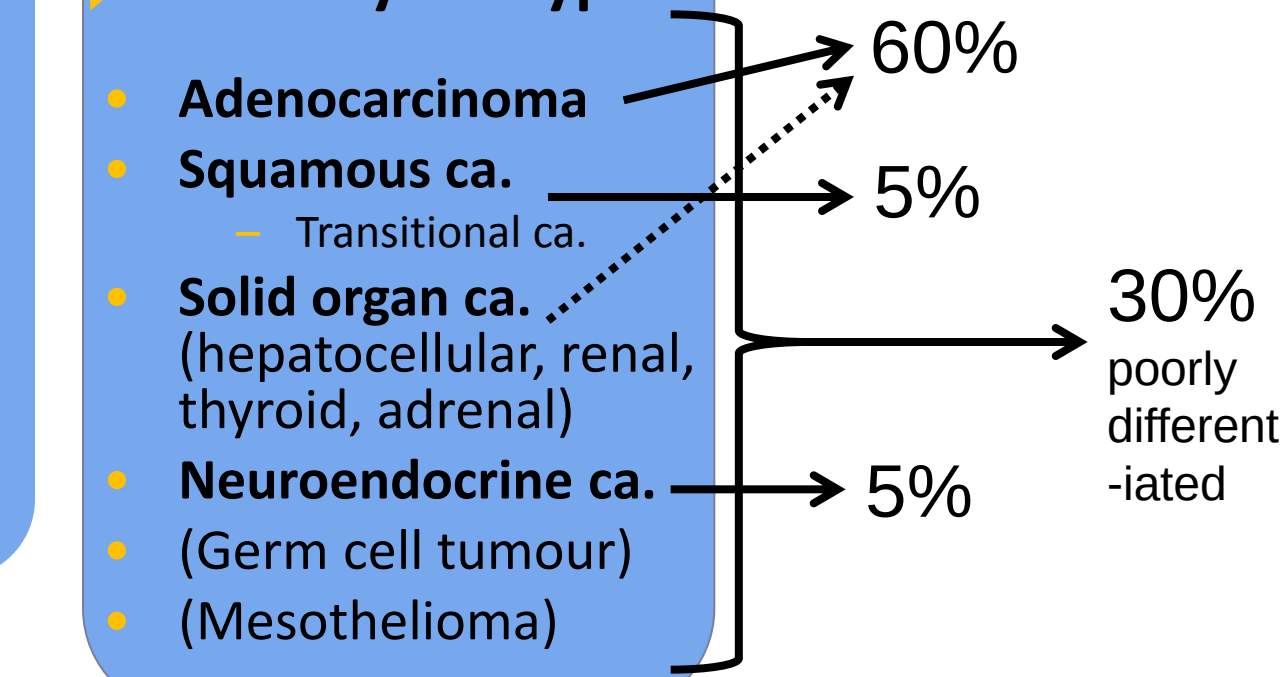

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**Step 3: if adeno-carcinoma, predict primary site(s)**

- Lung
- Pancreas
- Colon
- Stomach
- Breast
- Ovary
- Prostate, etc

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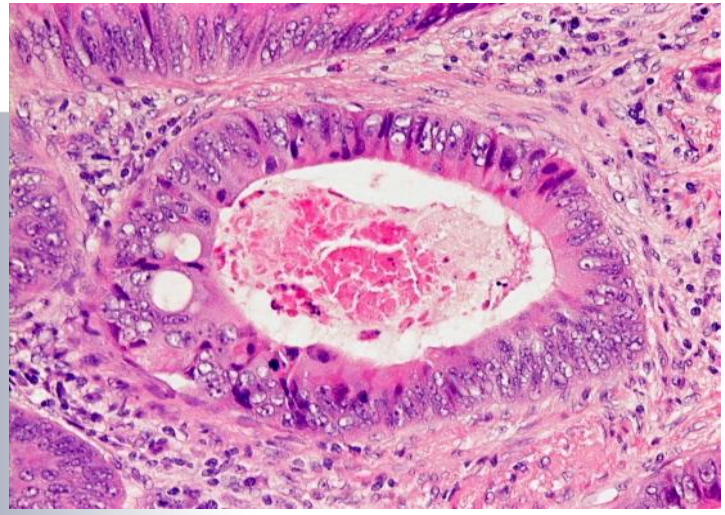
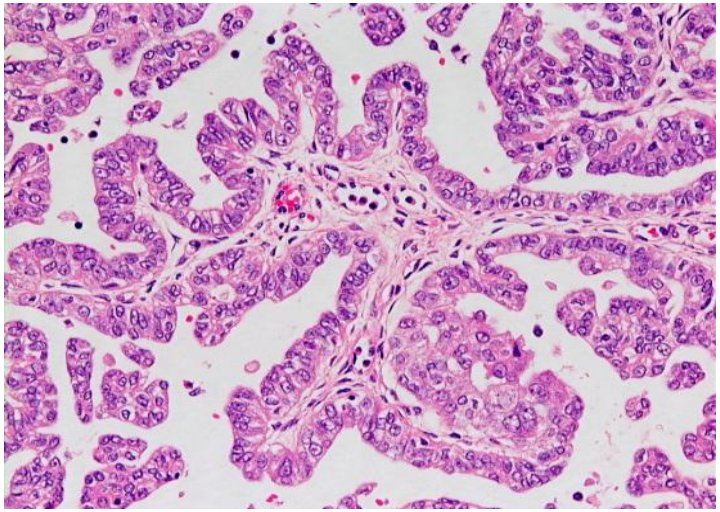
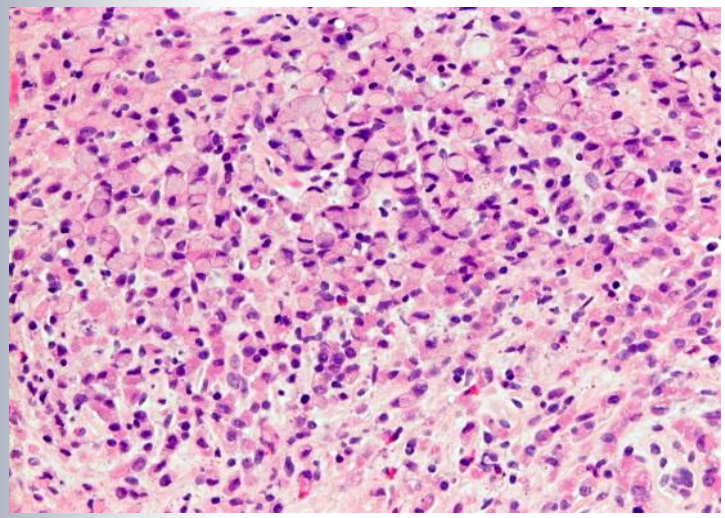
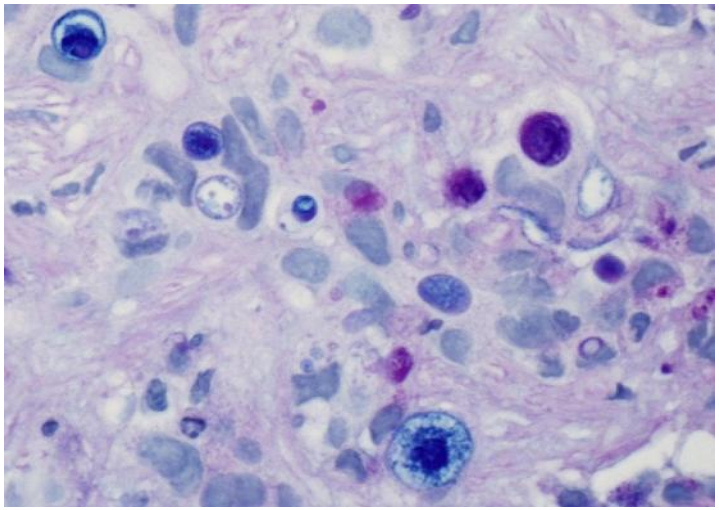
# Diagnostic approach: 3. If adenocarcinoma, predict primary site if possible







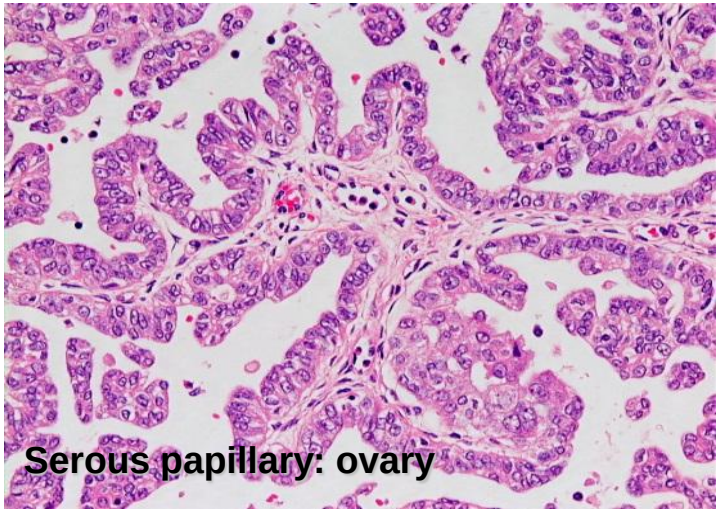


H&E morphology alone can predict primary site in up to 50% of cases

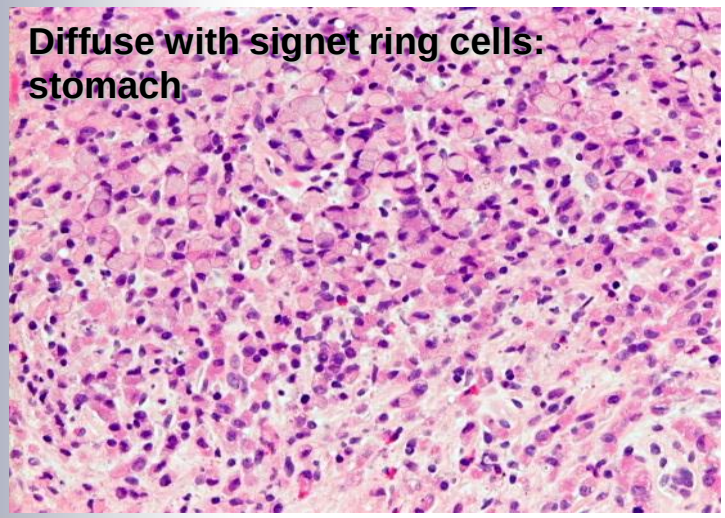
(Sheahan. Am J Clin Pathol 1993)

# Diagnostic approach: 3. If adenocarcinoma, predict primary site if possible

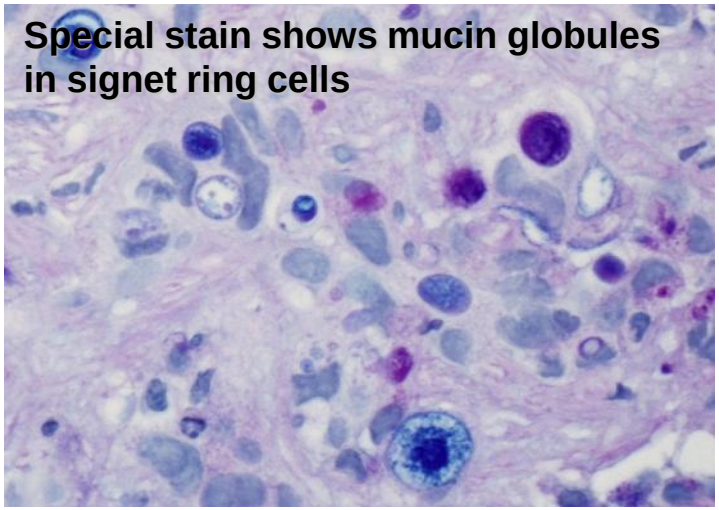
**“Dirty necrosis”: colon**



**Serous papillary: ovary**



**Diffuse with signet ring cells: stomach**



**Special stain shows mucin globules in signet ring cells**

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(Sheahan. Am J Clin Pathol 1993)

University of Glasgow

# IHC: Step 3: If adenocarcinoma, predict possible primary site(s)

| | PSA or NKX3.1 | TTF1 or Napsin A | GCDFP-15 or mammaglobin | WT1 | PAX8 | ER | CA125 | Meso-thelin | CK7 | CDX2 and/or CK20 |
|---|---|---|---|---|---|---|---|---|---|---|
| **Prostate** | + | - | - | - | - | - | - | - | - | - |
| **Lung** | - | + | - | - | - | - | -/+ | -/+ | + | - |
| **Breast** | - | - | +/- | - | - | +/- | -/+ | - | + | - |
| **Ovary serous** | - | - | - | + | + | +/- | + | + | + | - |
| **Ovary mucinous** | - | - | - | - | -/+ | -/+ | -/+ | -/+ | -/+ | -/+ |
| **Pancreas** | - | - | - | - | | - | +/- | +/- | + | -/+ |
| **Stomach** | - | - | - | - | | - | - | -/+ | +/- | -/+ |
| **Colon** | - | - | - | - | | - | - | - | -/+ | + |

+ = 90% or more, +/- = 50-90%, -/+ = 10-50%, - = 10% or less

Updated from Dennis, Clin Cancer Res 2005; 11(10):3766-3772.

# Performance & practice of pathology in CUP

- CUP specimens are usually small needle biopsies or cytology specimens
  - May limit testing possible
- When do we biopsy metastases: early or late during initial investigation?
- Practice varies between pathologists and between cases
  - Some pathologists usually diagnose confidently on morphology alone, others routinely add IHC

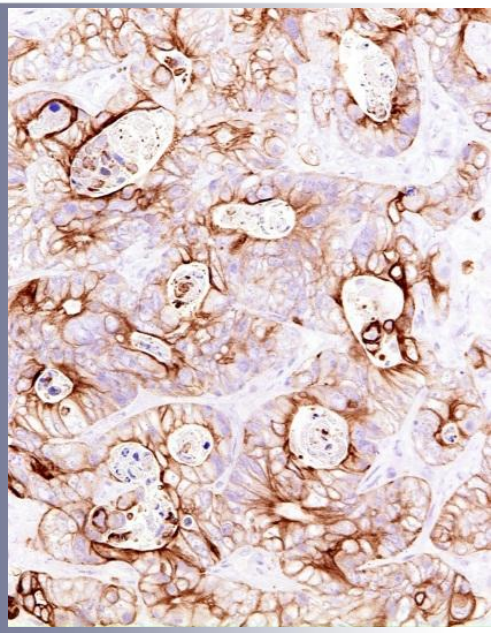
Image from **Royal College of Pathologists**

# Performance & practice of IHC esp. in CUP


- IHC is subjective: technical performance and microscopic interpretation varies (as does actual tissue expression), so IHC biomarkers used in panels
- IHC is selective: often limited tissue & time so only few biomarkers can be tested (7-8 is usual in CUP)
- In CUP, one barrier to correct tumour classification is simply not selecting & testing with most appropriate markers
  - Important for pathologist to request / apply the right biomarkers

Image from Wellcome Images; Weiss et al 2012, Schroeder et al 2012

# Can we quantify performance of current pathology incl. IHC in CUP classification?

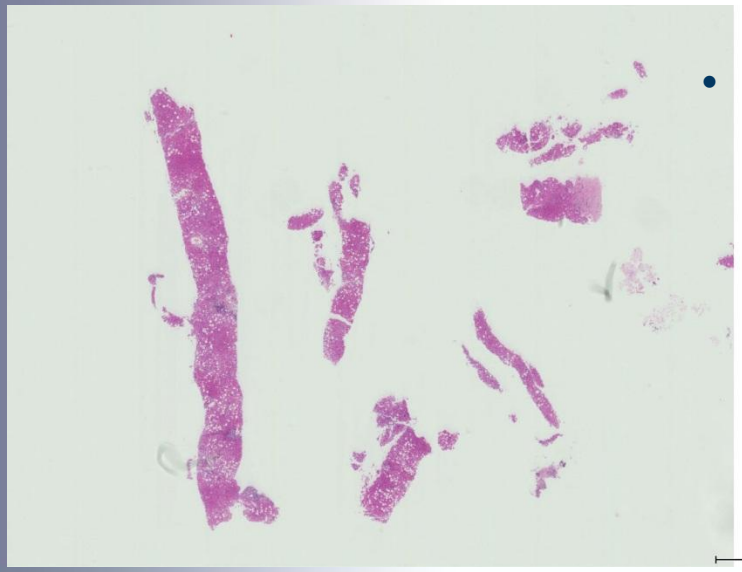


- 5-6 studies identified in meta-analysis
  - (Difficult to study by definition)
- Sensitivity of IHC panels was consistent:
  - 82% in mixed primary and metastatic tumours
  - 66-70% in metastases alone
- Sets baseline for comparison

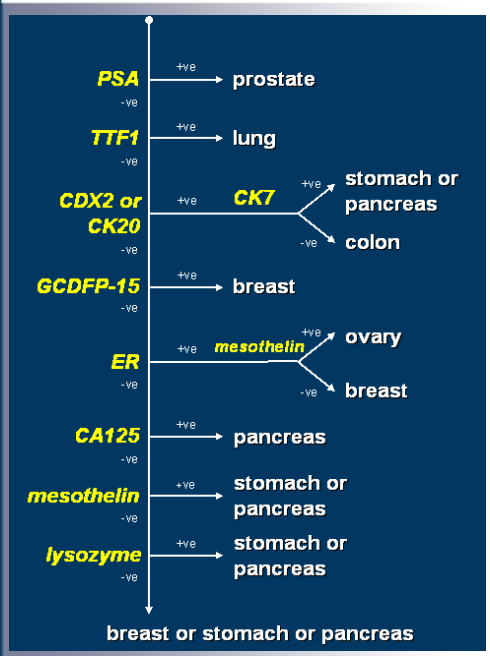
Anderson et al 2010, Weiss et al 2012, Sanden et al 2012

# Diagnostic difficulties: “tissue issues”

- After morphology, with IHC if needed, common diagnostic difficulties in classical tumour typing are with:
  - Limited viable tissue (“general tissue issues”)
    - **Small samples** especially if further testing requested at end of pathology processes
    - **Necrotic samples** (tumour tissue “dead”)



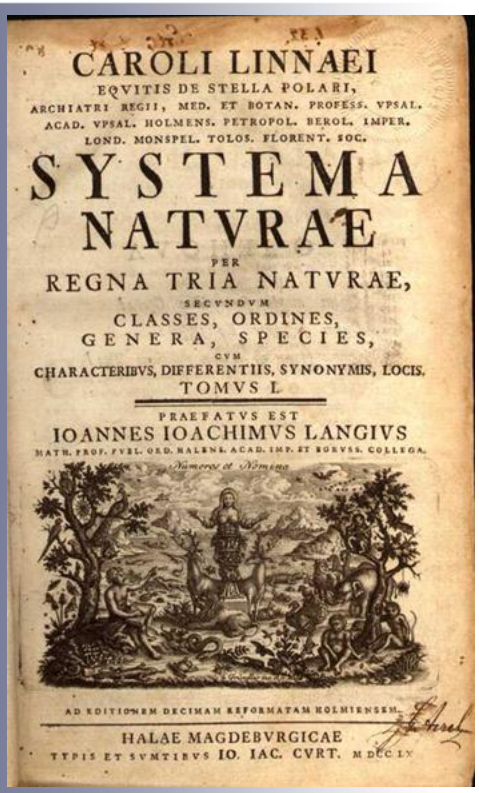
# Diagnostic difficulties: difficult morphology




- After morphology, with IHC if needed, common diagnostic difficulties in classical tumour typing for CUP are with:
  - "Morphology issues"
    - Very poorly differentiated or undifferentiated ("anaplastic") cancers
    - Adenocarcinoma, even well-differentiated, without obvious primary site after testing
      - Pancreatico-biliary including cholangiocarcinoma
      - Gastro-oesophageal
      - Ovarian mucinous
      - Atypical tumours from lung, breast etc
- These are unmet clinical needs for e.g. molecular profiling to address

Dennis, Clin Cancer Res 2005; 11(10):3766-3772.

# Outline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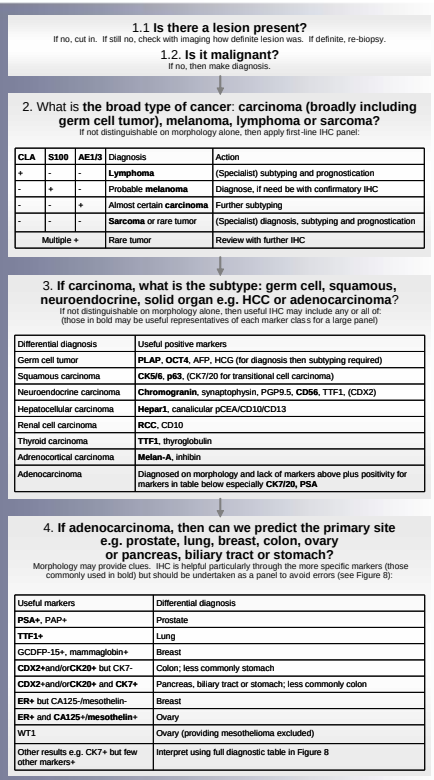
AD EDITIONEM DECIMAM REFORMATAM HOLMIENSEM
HALAE MAGDEBVRGICAE
TYPIS ET SVMTIBVS IO. IAC. CVRT. MDCCLX

- Pathological approach to cancer classification in general; and thus…
- Pathological approach to CUP incl. IHC
  - **Developments**
- **Molecular pathology for CUP**

sourced from http://bibbild.abo.fi/Linneana/ category:books

# Standardised & multi-disciplinary approaches



1.1 **Is there a lesion present?**
If no, cut in. If still no, check with imaging how definite lesion was. If definite, re-biopsy.

1.2. **Is it malignant?**
If no, then make diagnosis.

2. What is **the broad type of cancer**: **carcinoma (broadly including germ cell tumor), melanoma, lymphoma or sarcoma?**
If not distinguishable on morphology alone, then apply first-line IHC panel:

| CLA | S100 | AE1/3 | Diagnosis | Action |
|---|---|---|---|---|
| + | - | - | **Lymphoma** | (Specialist) subtyping and prognostication |
| - | + | - | Probable **melanoma** | Diagnose, if need be with confirmatory IHC |
| - | - | + | Almost certain **carcinoma** | Further subtyping |
| - | - | - | **Sarcoma** or rare tumor | (Specialist) diagnosis, subtyping and prognostication |
| Multiple + | | | Rare tumor | Review with further IHC |

3. **If carcinoma, what is the subtype: germ cell, squamous, neuroendocrine, solid organ e.g. HCC or adenocarcinoma?**
If not distinguishable on morphology alone, then useful IHC may include any or all of: (those in bold may be useful representatives of each marker class for a large panel)

| Differential diagnosis | Useful positive markers |
|---|---|
| Germ cell tumor | **PLAP**, **OCT4**, AFP, HCG (for diagnosis then subtyping required) |
| Squamous carcinoma | **CK5/6**, **p63**, (CK7/20 for transitional cell carcinoma) |
| Neuroendocrine carcinoma | **Chromogranin**, synaptophysin, PGP9.5, **CD56**, TTF1, (CDX2) |
| Hepatocellular carcinoma | **Hepar1**, canalicular pCEA/CD10/CD13 |
| Renal cell carcinoma | **RCC**, CD10 |
| Thyroid carcinoma | **TTF1**, thyroglobulin |
| Adrenocortical carcinoma | **Melan-A**, inhibin |
| Adenocarcinoma | Diagnosed on morphology and lack of markers above plus positivity for markers in table below especially **CK7/20, PSA** |

4. **If adenocarcinoma, then can we predict the primary site e.g. prostate, lung, breast, colon, ovary or pancreas, biliary tract or stomach?**
Morphology may provide clues. IHC is helpful particularly through the more specific markers (those commonly used in bold) but should be undertaken as a panel to avoid errors (see Figure 8):

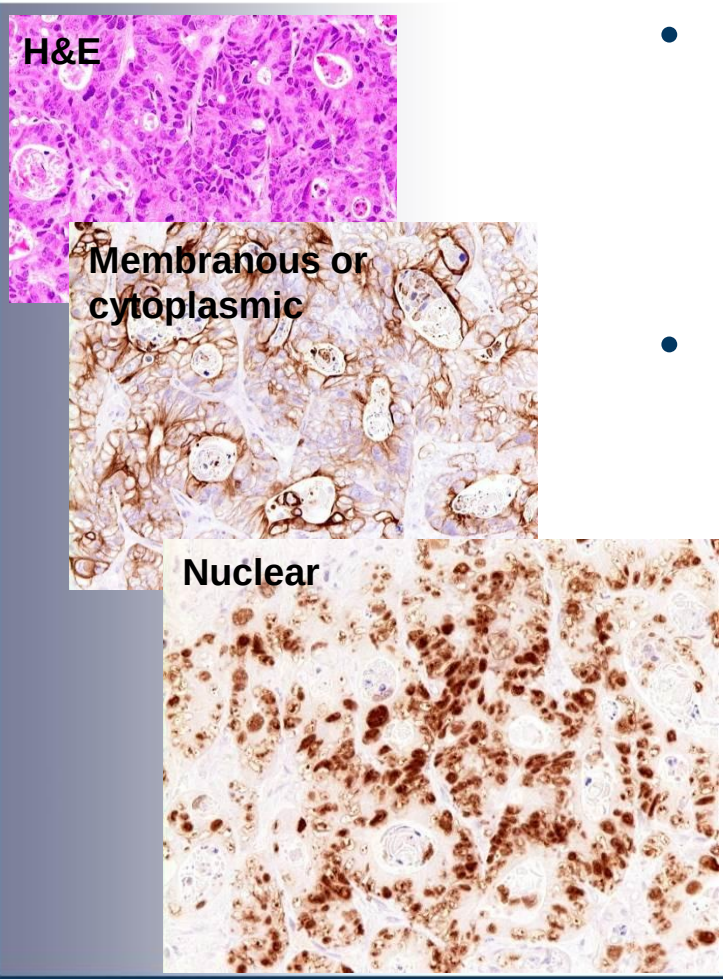
| Useful markers | Differential diagnosis |
|---|---|
| **PSA+**, PAP+ | Prostate |
| **TTF1+** | Lung |
| GCDFP-15+, mammaglobin+ | Breast |
| **CDX2+**and/or**CK20+** but CK7- | Colon; less commonly stomach |
| **CDX2+**and/or**CK20+** and **CK7+** | Pancreas, biliary tract or stomach; less commonly colon |
| **ER+** but CA125-/mesothelin- | Breast |
| **ER+** and **CA125+/mesothelin+** | Ovary |
| WT1 | Ovary (providing mesothelioma excluded) |
| Other results e.g. CK7+ but few other markers+ | Interpret using full diagnostic table in Figure 8 |

**e.g. flowcharts**

- "CUP" is a diagnosis of exclusion, at the **end** of pathological work-up
- In difficult cases, e.g. eventual CUP, systematic approach ensures all possibilities to be considered
- So most appropriate IHC markers can be requested (early) and assessed
  - To lead to tumour classification
- Guidelines & standards help e.g. NICE, ESMO, Peer Review, developing RCPath
- In clinical context: **MDT**

**Oien 2009 Semin Oncol.**

# New IHC biomarkers




- Emphasis on (nuclear) transcription factors
  - c.f. cytoplasmic and membranous proteins
- Examples
  - PAX8: ovary, kidney, thyroid
  - GATA3: breast & transitional carcinomas
  - SATB2: colorectal & renal
  - SF1: adrenal cortical carcinoma
  - (Arginase-1, uroplakin 2)

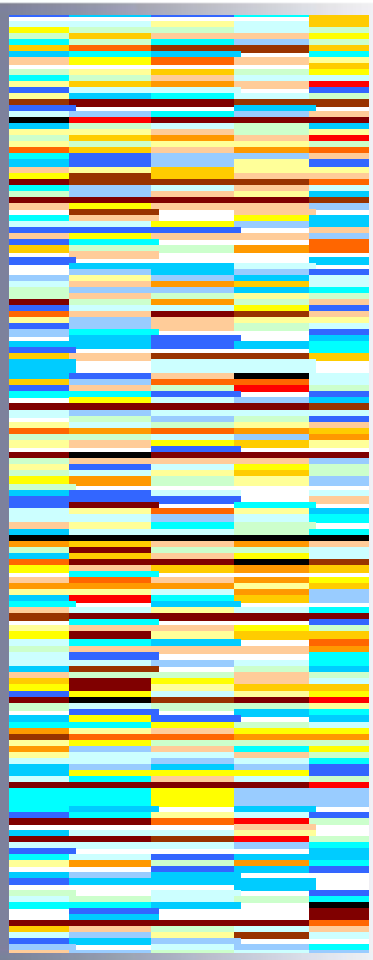
Connor & Hornick, Adv Anat Pathol 2015;22:149–167

# Molecular profiling in CUP

- Large-scale profiling for CUP achieved at mRNA, miRNA, DNA and epigenetic levels
  - Molecular profiling for primary site esp. mRNA/miRNA
    - Augments existing classification?
  - Molecular analysis for actionable mutations
    - New classification/taxonomy

Image from **Royal College of Pathologists; Pillai et al 2011, Stancel et al 2012, Erlander et al 2011, Meiri et al 2012**

# Molecular profiling for primary site: rationale

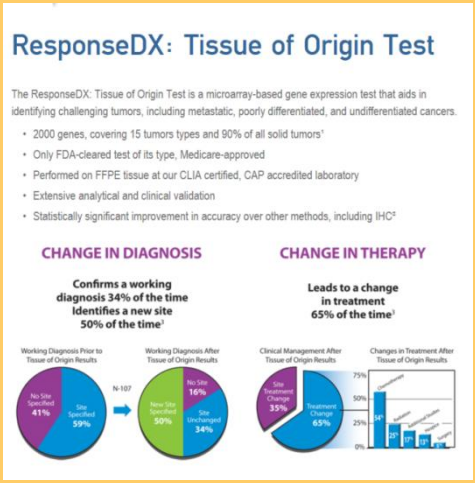


- “Different tissue types have distinct RNA profiles” (…or protein…etc)
- Yield tumour type, site and/or subtype i.e. classic taxonomy
- IHC and mRNA molecular profiling use similar tissue-specific genes:
  - Molecular profiling tests more genes and may be less subjective

Greco 2014, Pillai et al 2011, Stancel et al 2012, Erlander et al 2011, Meiri et al 2012, Oien 2012

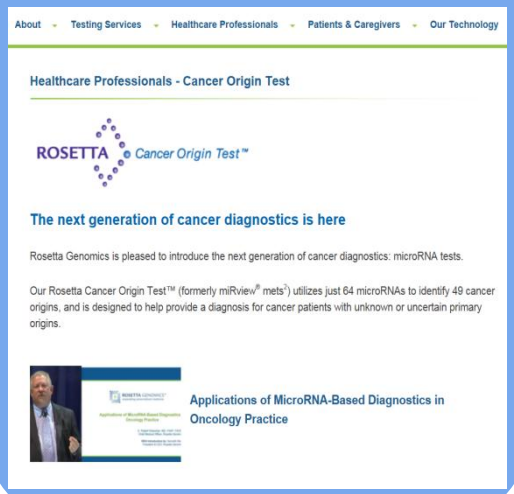
# Three CUP RNA tests commercially available

## Tissue of Origin (TOO) test (Response Genetics, previously PathWorks Diagnostics)




## Cancer Type ID (CTID) (bio-Theranostics)

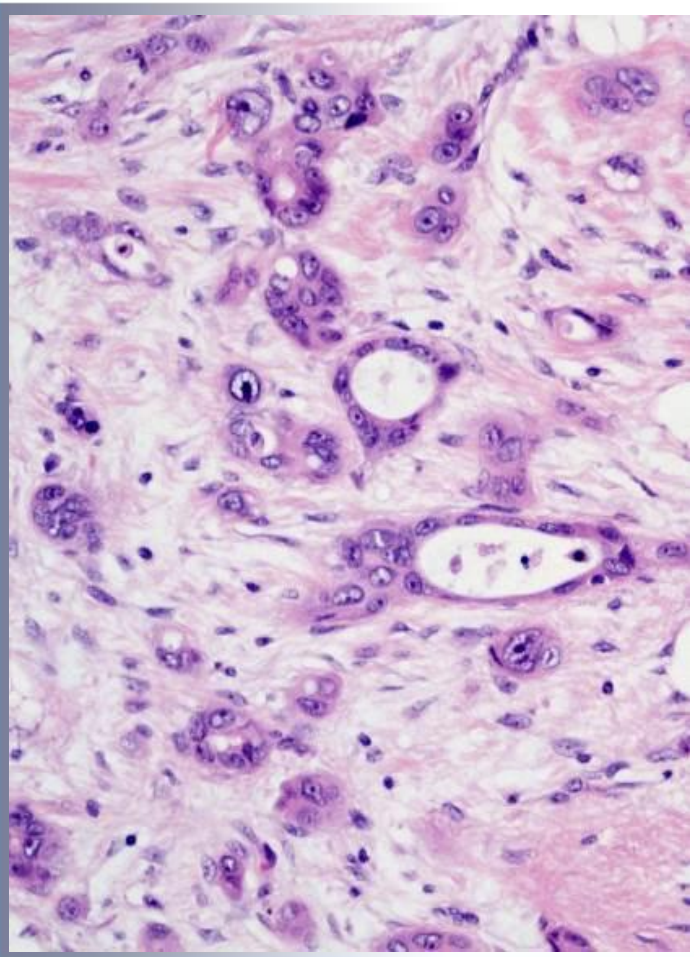

## miRview mets2 (Rosetta Genomics)




http://rosettagenomics.com/

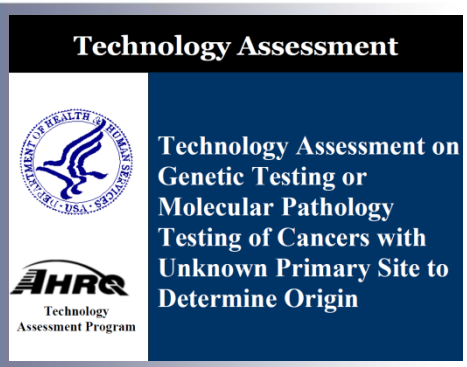
http://www.responsegenetics.com/products-services/tissue-of-origin-testing/

# Molecular profiling for primary site: results



- 10% of tests yield no result
- Tumours difficult for morphology and IHC often also difficult for molecular profiling
- In poorly differentiated tumours, molecular profiling *may*:
  - Out-perform IHC by 10-20%
  - Change diagnosis in up to 50%
  - Affect patient management in most?
- Interpret in context of clinical and pathological findings

# Technology Assessment on CUP Tests




- "Clinical accuracy of all three tests is similar: 85-88%. Test accuracy in CUP cases is not easily determined, because actual TOO is not identified in most cases.
- The evidence that the tests contribute to identifying a TOO is moderate.
- We do not have **sufficient evidence to assess the effect of the tests on treatment decision and outcomes."**
- Which should be **focus** of future research
- Utility, funding?

2013 http://www.ncbi.nlm.nih.gov/pubmedhealth/PMH0073073/pdf/TOC.pdf

# Molecular analysis for actionable mutations

- New classification/taxonomy
- Approach?  Panel?
  - e.g. FoundationOne, Caris, BioTheranostics

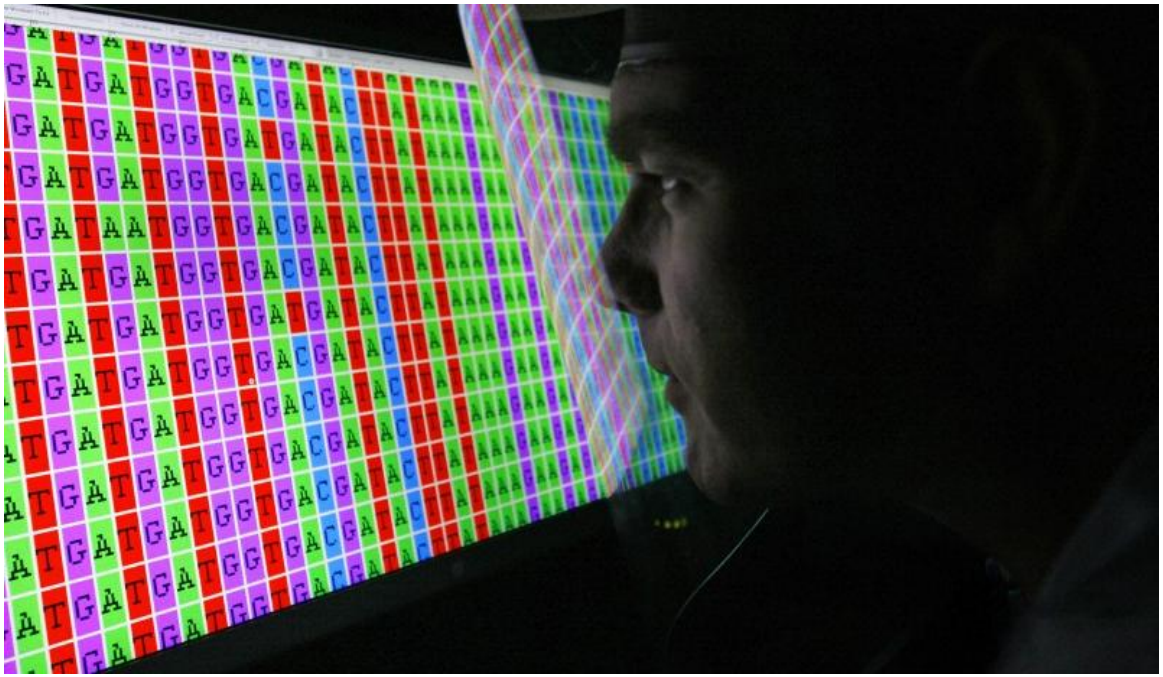


Image from **Royal College of Pathologists**

# PROSPECTIVE IDENTIFICATION OF POTENTIALLY ACTIONABLE MOLECULAR ALTERATIONS IN CANCERS OF UNKNOWN PRIMARY

Memorial Sloan Kettering Cancer Center

**Anna M. Varghese**, David Hyman MD, Debyani Chakravarty PhD, Ahmet Zehir PhD, Efsevia Vakiani MD PhD, David Klimstra MD, Marc Ladanyi MD, Michael F. Berger PhD, David B. Solit MD, and Leonard B. Saltz MD

**Memorial Sloan Kettering Cancer Center, New York, NY**

**Abstract # 4110**

## Background

•Cancers of unknown primary (CUP) affect approximately 31,000 people annually in the United States.[1]
•Patients with CUPs have a poor prognosis with survival ranging from 6-16 months. [2 3]
•Recent findings have demonstrated that up to 85% of patients with CUPs have "actionable" alterations.[4 5]
•We hypothesize that a substantial percentage of tumors from patients with CUP could harbor potentially actionable and clinically relevant alterations.

## Objective

**To perform prospective, comprehensive genomic characterization of confirmed cancers of unknown primary in order to identify actionable alterations.**

## Methods

•MSK-IMPACT is a comprehensive molecular profiling platform performed in a CLIA-compliant laboratory using targeted deep sequencing of 341 cancer-associated genes to identify sequence mutations, copy number alterations, and select rearrangements.[6]
•We identified patients with CUP who underwent MSK-IMPACT testing at MSKCC from 01/2014 through 04/2015.
•We excluded patients with a likely primary disease site based on subsequent pathologic and radiographic tests.
•We collected tumor histology and defined potentially actionable alterations as those for which FDA-approved or investigational targeted agents exist with varying levels of evidence.

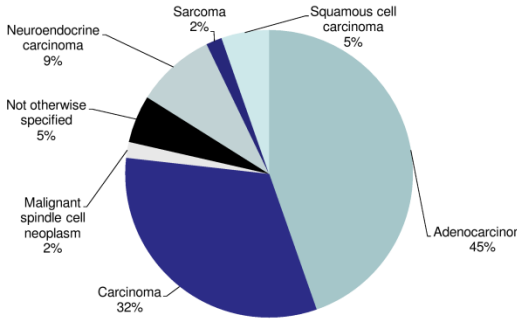
## Results

### Clinical characteristics of patients with CUP

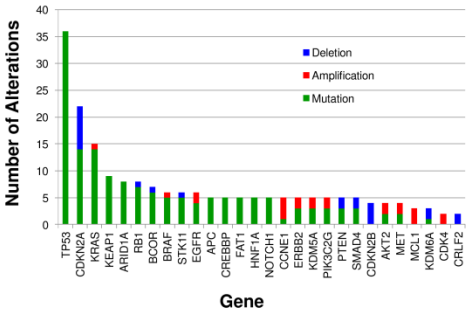
| | N | % |
|---|---|---|
| **Total** | 56 | |
| **Sex – M / F** | 32 / 24 | 57 / 43 |
| **Race** | | |
| **White / Black / Asian** | 47 / 2 / 1 | 84 / 4 / 1 |
| **Unknown** | 6 | 11 |
| **ECOG status** | | |
| **0** | 13 | 23 |
| **1** | 33 | 60 |
| **2** | 1 | 1 |
| **Unknown** | 9 | 16 |
| **Median age at dx (yrs)** | 66 (range: 21 – 83) | |

## Results

### Histologic characteristics of patients with CUP




### Genetic alterations in CUPs

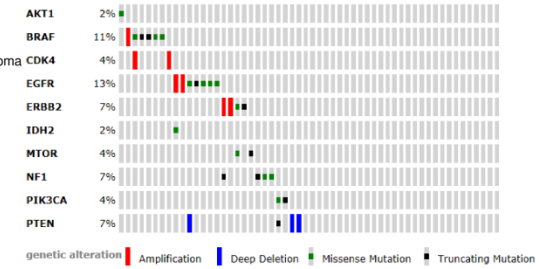



## Results

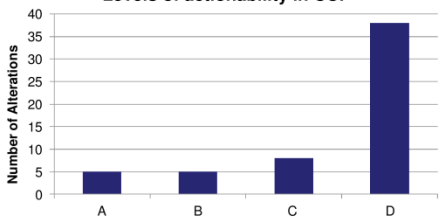
### Actionability in CUP

| Level | Definition | Examples |
|---|---|---|
| A | FDA-approved biomarker in another indication | *BRAF* V600E |
| B | Clinical evidence potentially links this biomarker to response | *PIK3CA* H1047R |
| C | Preclinical evidence potentially links this biomarker to response | *ERBB2* S310F |
| D | No preclinical or clinical evidence to link this biomarker to response | *TP53* alterations |

### Prevalence of potentially actionable alterations in CUP




### Levels of actionability in CUP




## Results

### Actionable alterations in CUP

| Level | Gene | Alteration | # | Clinical Trial Available? |
|---|---|---|---|---|
| A | *BRAF* | V600E | 3 | Y |
| | *ERBB2* | Amplification | 2 | N |
| B | *BRAF* | G469V | 1 | N |
| | *CDK4* | Amplification | 1 | N |
| | *IDH2* | R172W | 1 | Y |
| | *PIK3CA* | E545K | 1 | Y |
| | | H1047R | 1 | Y |
| C | *AKT1* | E17K | 1 | Y |
| | *EGFR* | Amplification | 1 | N |
| | *ERBB2* | S310F | 1 | Y |
| | *NF1* | D618fs | 1 | Y |
| | *PTEN* | Deletion | 3 | Y |
| | *MTOR* | D1468Y | 1 | N |

### Clinical relevance for selected patients

•80yo male with *BRAF* V600E-mutant squamous cell CUP received vemurafenib with 4 months stable disease.
•50yo female with *AKT1* E17K-mutant carcinoma of unknown primary recently began treatment on a clinical trial targeting her tumor's AKT1 alteration, awaiting restaging imaging.

## Conclusions

•32% of CUPs harbor potentially actionable alterations.

•The implications of these potentially actionable alterations on treatment choices and clinical outcomes remain unknown.

## References

1.Siegel et al. *CA: a cancer journal for clinicians.*2015.
2.Varadhachary et al. *New England Journal of Medicine.* 2014.
3.Pavlidis et al. *Eur J Cancer.* 2003
4.Tothill et al. *Journal of Pathology.* 2013
5.Ross et al. *JAMA Oncology.* 2015
6. Cheng et al. *J Mol Diagn.* 2015

## Conclusions

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•The implications of these potentially actionable alterations on treatment choices and clinical outcomes remain unknown.

**From: Comprehensive Genomic Profiling of Carcinoma of Unknown Primary Site: New Routes to Targeted Therapies**

**JAMA Oncol. 2015;1(1):40-49. doi:10.1001/jamaoncol.2014.216**

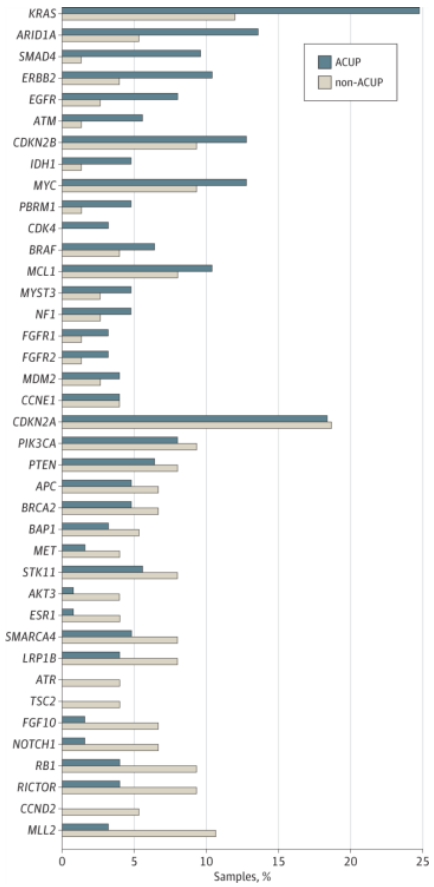
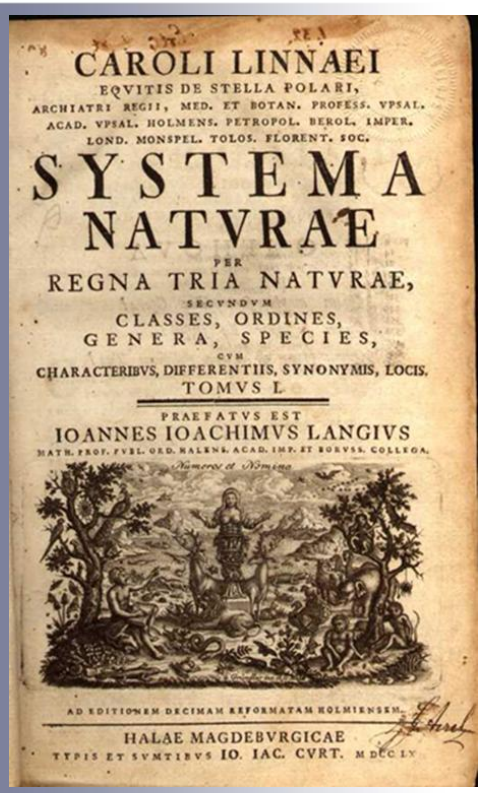



**Figure Legend:**

**Alteration Frequency of Key Genes in Cases of Adenocarcinoma of Unknown Primary Site (ACUP) vs non-ACUP**

# Conclusions: Pathology incl. IHC in CUP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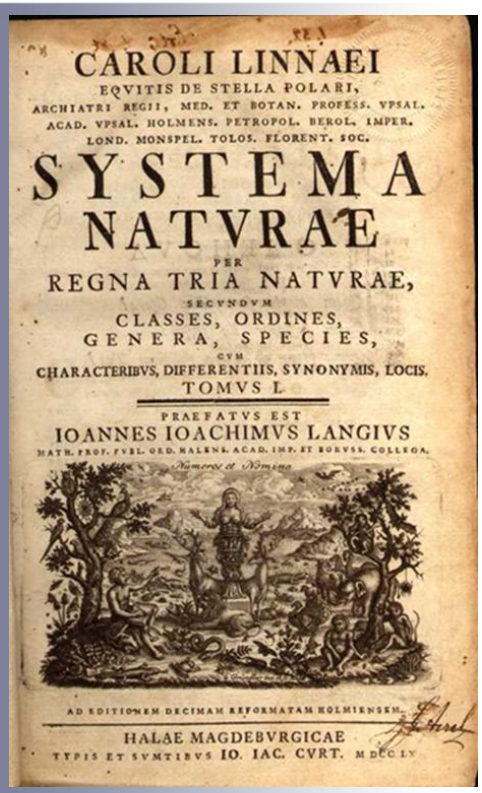
HALAE MAGDEBVRGICAE
TYPIS ET SVMTIBVS IO. IAC. CVRT. MDCCLV

- Pathology, with IHC if needed, remains "gold standard" in tumour classification, especially where:
  - Tumour has obvious likely primary and/or
  - Tumour is at least moderately differentiated and/or
  - IHC results are classical and/or
  - The clinical context is appropriate
- Optimal pathology enabled by:
  - Standardised approach incl. IHC
  - MDT approach to enable full clinical context and knowledge of potential treatments

# Conclusions: Unmet clinical needs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HALAE MAGDEBVRGICAE
TYPIS ET SVMTIBVS IO. IAC. CVRT. MDCCLV

- Poorly differentiated cancers and adeno-carcinomas without obvious primary, especially where:
  - Current pathology equivocal for management or conflicting with clinical context
  - Diagnosis either truly unknown or includes multiple possible differentials
  - Limited tissue or **time**?
- Better prediction of treatment benefit
- Discussion: role of molecular pathology for:
  - Site/type
  - Actionable mutations

# Acknowledgements


- Thanks to:
  - Dr Jayne Dennis
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  - Prof Nicol Keith
  - Prof Jeff Evans
  - Profs Andrew Biankin & Sean Grimmond and colleagues
- Cancer Research UK and University of Glasgow for funding: but this presentation is my personal opinion