Reporting of carcinoma of unknown primary tumour (CUP)

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with grateful thanks to

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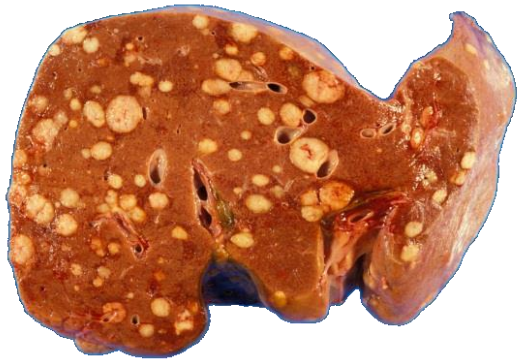
Royal College of Pathologists - Cancer datasets 30 March 2017

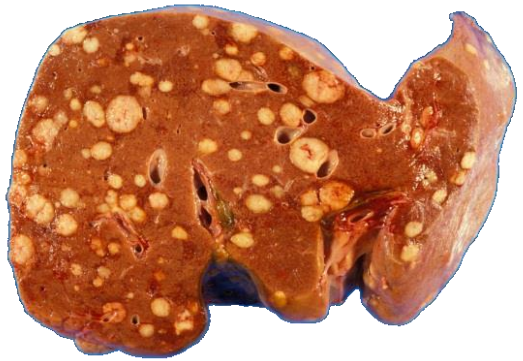
Cancer of Unknown Primary: The Problem

- Most cancers present at their primary site
- **10-15%** present as metastasis
  - In two-thirds or more, primary becomes evident
  - In up to one-third (**5%**), primary site is not found and this becomes CUP
- Cancers of unknown primary site (CUPs) are a heterogeneous group of **metastatic tumours** for which a standardised diagnostic work-up fails to identify site of origin at time of diagnosis
  - Definition: ESMO Clinical Practice Guidelines (European Society of Medical Oncology)

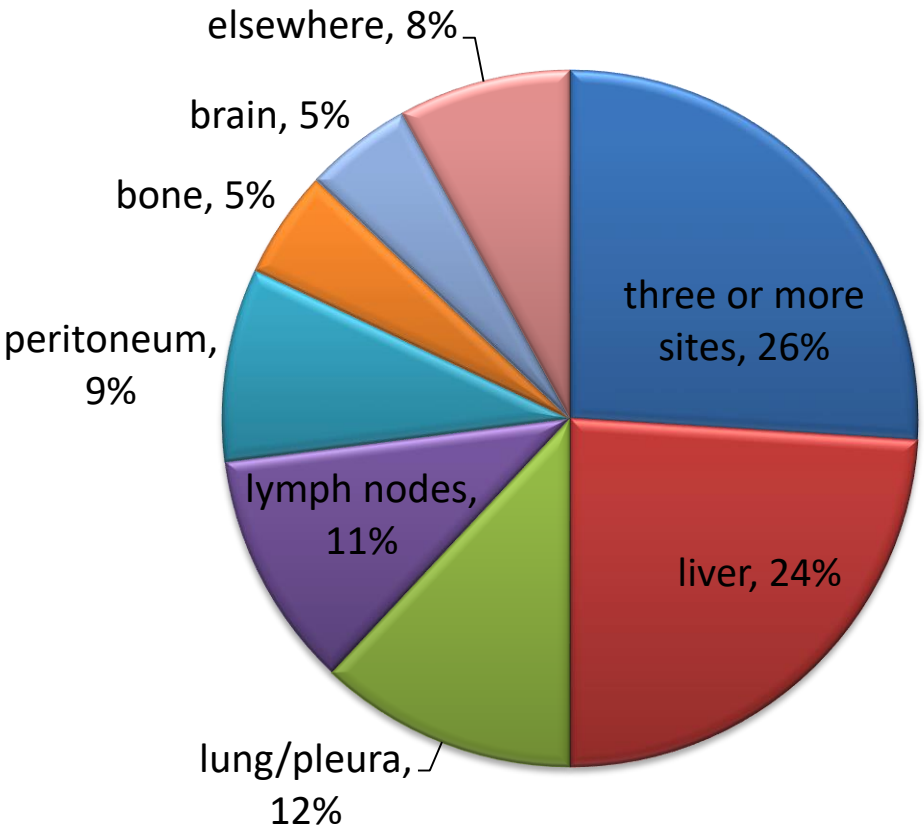


CUP Epidemiology

- Up to 1980's, CUP made up **10-15%** of patients referred to oncology
- Site of origin now more often identified but CUP still forms **3-5%** of all malignancies
- Worldwide, CUP is **fourth** most common cause of cancer death
- Median age 60 with 53% M: 47% F
- Median survival: 3-10 months historically



CUP common presenting sites for metastasis

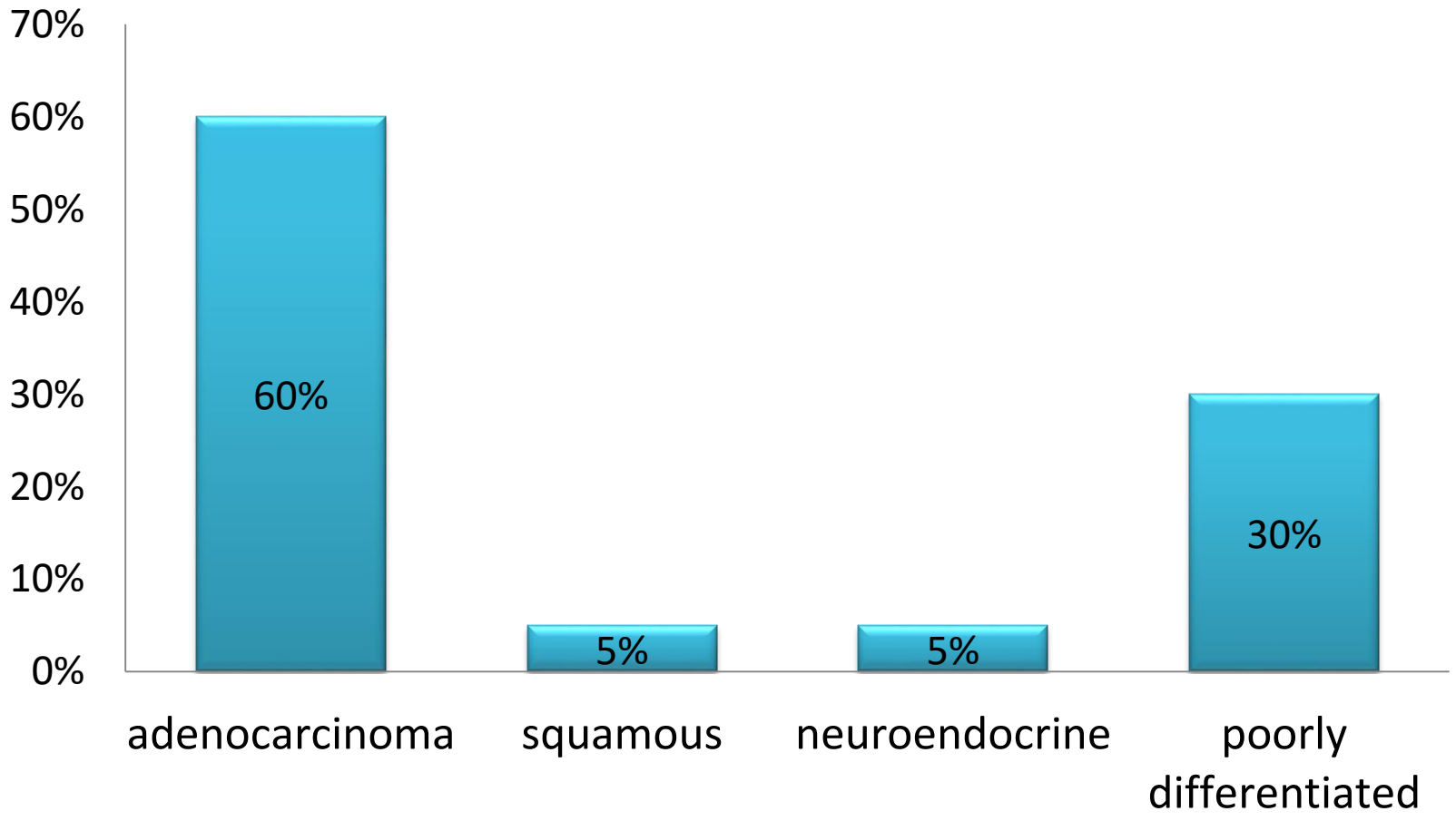


- Solid organs: liver, lung, bone & brain
- Lymph nodes: cervical, supra-clavicular, axillary, mediastinal/ retroperitoneal and inguinal
- Serous cavities: peritoneal and pleural
- **(i.e. common sites of metastasis overall)**

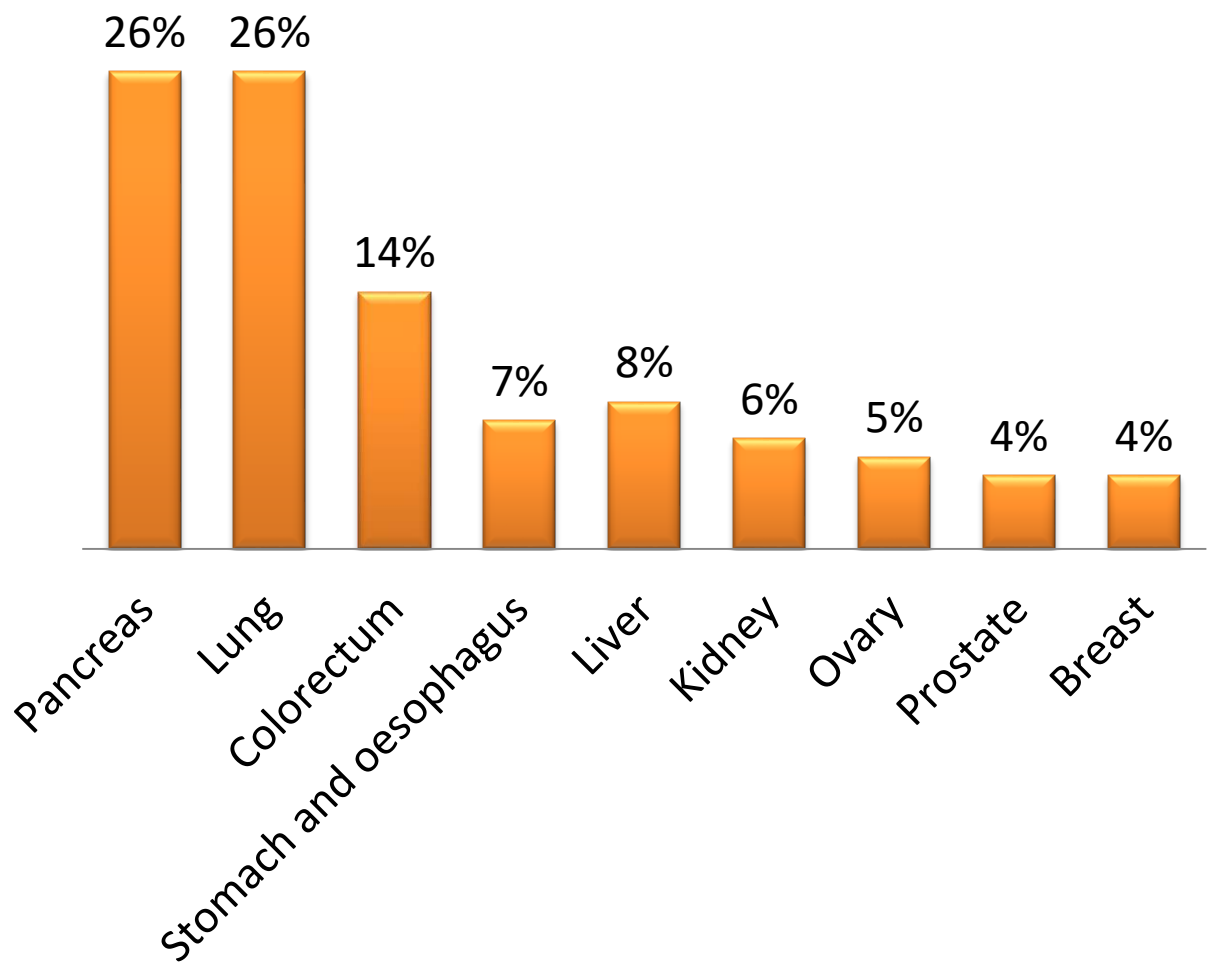
CUP tumour types

- Most clinical studies of CUP exclude lymphoma, metastatic melanoma and metastatic sarcoma
  - And often require histological confirmation
- Therefore **cancer** of unknown primary generally equates to **carcinoma** of unknown primary
- Other main tumour types need considered and excluded during pathological work-up
- CUP is a **“diagnosis of exclusion”**

CUP common histological sub-types



CUP common sites of origin at autopsy



- Historically, after CUP diagnosis, primary site identified only in:
  - <20–25% in life;
  - 30-80% at autopsy
- Gives (historical) common sites of origin

CUP: classification for clinical benefit

- Despite CUP representing metastatic malignancy, outlook is not uniformly poor
- Clinical subsets with better or worse outcomes identified, based on:
  - Histopathological type of tumour:
    - (Lymphoma), neuroendocrine, germ cell
  - Anatomical site:
    - LN = good exc. supraclav
  - Number of metastatic sites involved
  - Overall performance status
- And with better or worse response to therapy

“Good Prognosis” or “Favourable” CUP Subsets

Favourable subset of CUP	Treatment as for equivalent stage of:
Squamous carcinoma in cervical LN	**Head and neck cancer** Locoregional therapy: surgery and/or irradiation
Squamous carcinoma in inguinal LN	**Genito-urinary tumour** Locoregional therapy: surgery and/or irradiation
Adenocarcinoma in axillary LN (female)	**Breast cancer** Locoregional then systemic therapy
Extragonadal **germ cell tumour** in LN or lung (male)	Poor prognosis germ cell tumour Platinum-based chemotherapy
Serous papillary adenocarcinoma in peritoneum (female)	Ovarian cancer Taxane/platinum-based chemotherapy
Neuroendocrine carcinoma	Platinum or paclitaxel/carboplatin based chemotherapy
Adenocarcinoma in bone with high PSA (male)	**Prostate cancer** with hormonal therapy
Single small metastasis in solid organ (liver, lung, brain)	Consider local treatment with resection and/or radiation, and/or systemic chemotherapy

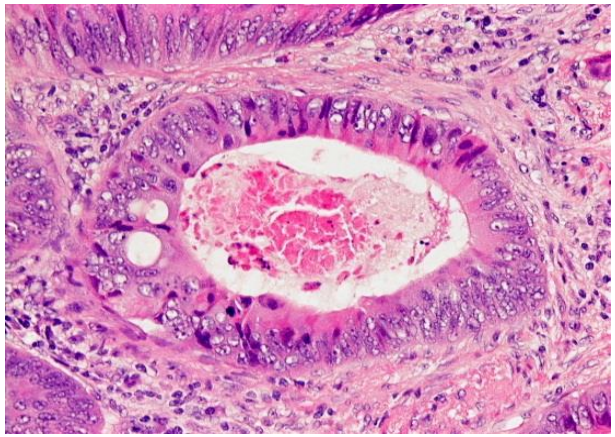
- CUP subsets sensitive to either loco-regional treatment or systemic chemotherapy, often with curative intent

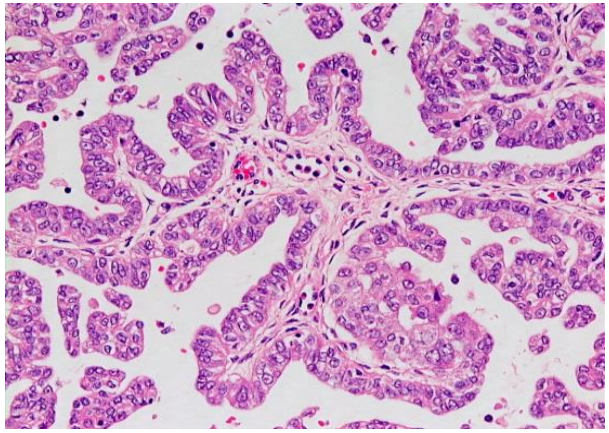
“Poor prognosis” or “unfavourable” CUP Subsets

- Multiple metastases in any one solid organ
- Malignant ascites with non-serous-papillary adenocarcinoma; and pleural effusion
- Most liver metastases except single colorectal-like deposit
- Most of these “unfavourable” tumours are adenocarcinomas

	Hormone	Anthra-cycline	Platinum	Taxane	5FU	Gem-citabine
Breast						
Colon						
Lung						
Ovary						
Pancreas						
Prostate						
Stomach						

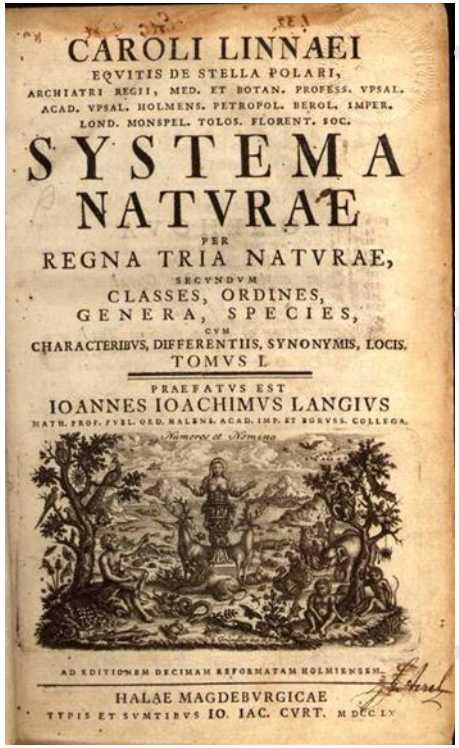
CUP Pathology: H&E





- “CUP” generally equates to carcinoma
- Other cancers need to be considered and excluded
- Cancer classification for type & site classically based on morphology
  - Resemblance to normal tissue counterpart
- For adenocarcinomas, H&E morphology alone can predict primary site in up to 50% of cases

Classification..or “reduction of uncertainty”...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LOND. MONSPEL. TOLOS. FLORENT. SOC.
SYSTEMA
NATVRAE
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REGNA TRIA NATVRAE,
SECVNDVM
CLASSES, ORDINES,
GENERA, SPECIES,
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CHARACTERIBVS, DIFFERENTIIS, SYNONYMIS, LOCIS.
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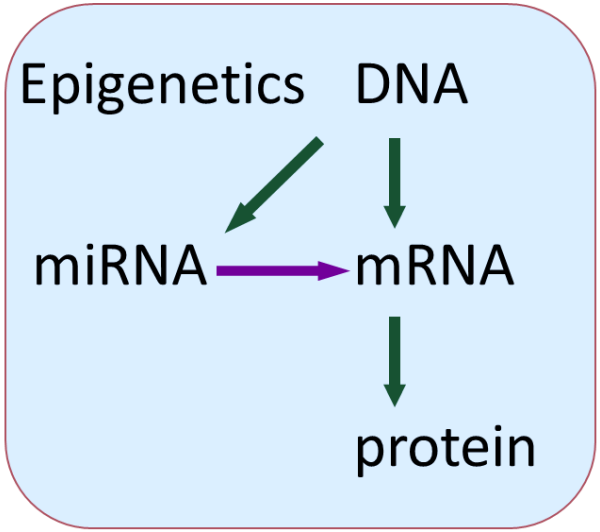
Numero et Nomine
AD EDITIONEM DECIMAM REFORMATAM HOLMIENSEM.
HALAE MAGDEBVRGICAE
TYPIS ET SVMTIBVS IO. IAC. CVRT. MDCCLX

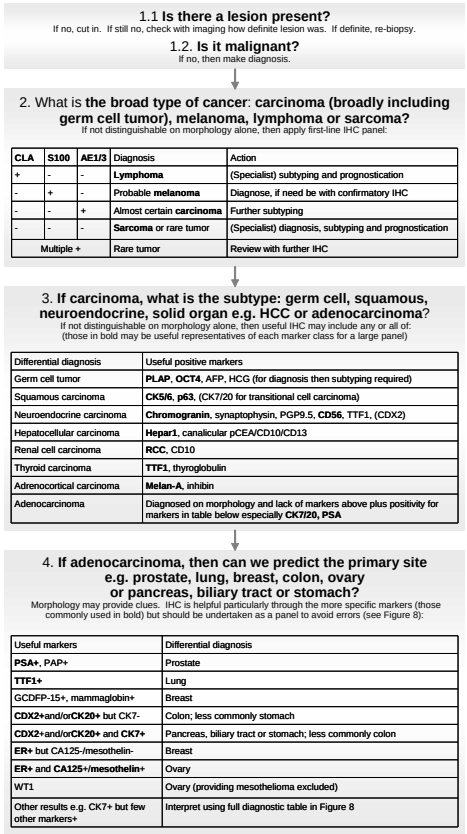
- In cancer pathology and CUP, our aim is to provide optimal cancer classification
- We’re trying to reduce uncertainty
  - For clinical colleagues and patients
  - On behalf of pathology
- Can we improve classification with more or better biomarkers?

Tissue-specific/restricted genes



- Morphology reflects gene expression
  - Around 12,000 genes are active in any one tissue type
  - Over 8,000 genes are widely expressed
- Minority of genes are **tissue-specific or tissue-restricted**, related to function i.e. differentiation
  - Regulatory genes in nucleus e.g. ER, TTF1, CDX2, PAX8
  - Protein products in cytoplasm or membrane e.g. PSA, CK7

Where does this fit in pathology workup?… Towards standardised approaches in CUP



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):

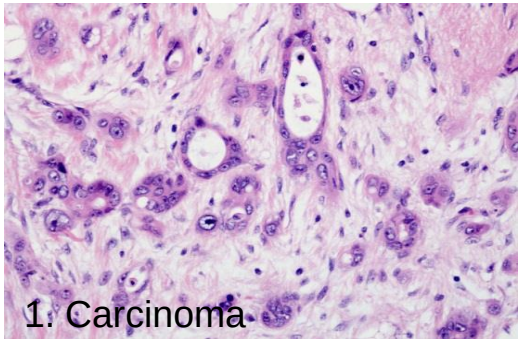
Useful markers	Differential diagnosis
PSA+, PAP+	Prostate
TTF1+	Lung
GCDFP-15+, mammaglobin+	Breast
CDX2+and/or**CK20+** but CK7-	Colon; less commonly stomach
CDX2+and/or**CK20+** and **CK7+**	Pancreas, biliary tract or stomach; less commonly colon
ER+ but CA125-/mesothelin-	Breast
ER+ and **CA125+/mesothelin+**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

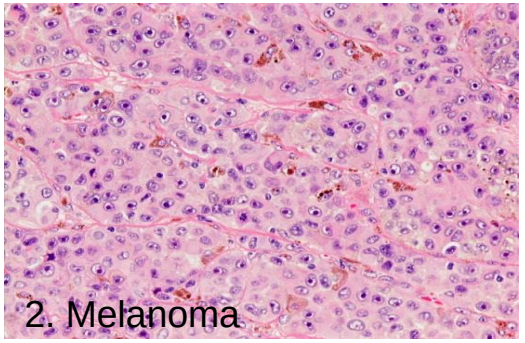
- Attempt to predict primary site is at end of pathology work-up, as part of diagnosis of exclusion to establish CUP
- Step-wise work-up is familiar to pathologists
  - Diagnostic decisions often based on H&E morphology alone and rather “black box”
  - Less familiar for early trainees, other clinicians and scientists therefore useful to describe explicitly
- In difficult cases, e.g. eventual CUP, systematic approach ensures all differential diagnoses considered
  - So the most appropriate IHC markers are used

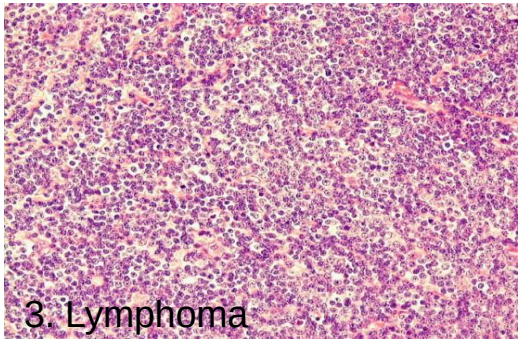
Classification of cancer including CUP: A stepwise pathological approach

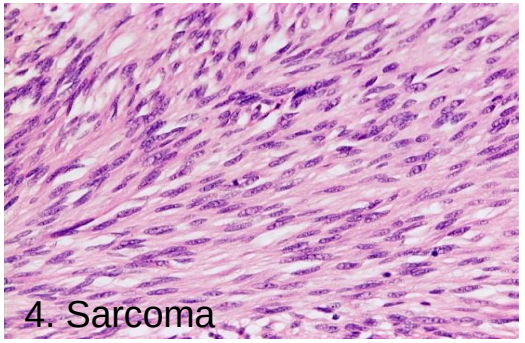
Step 1: identify broad cancer type

- Carcinoma
- Melanoma
- Lymphoma/ leukaemia
- Sarcoma
- (Neuroglial tumours)









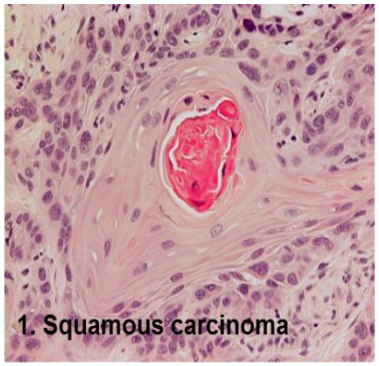
Immunohistochemistry for CUP: Step 1: identify broad cancer type

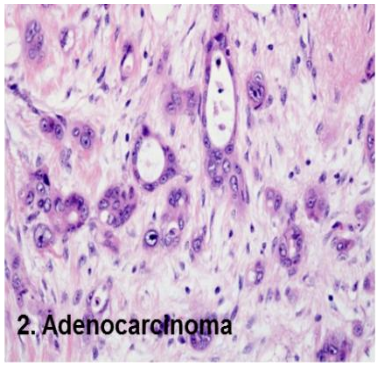
Carcinoma	**(Pan-)Cytokeratins** and other epithelial markers e.g. **AE1/3**, CK7, CK20, CK5, EMA
Melanoma	**S100**, Melan-A, HMB45
Lymphoma/ leukaemia	**CLA**, CD20, CD3, CD138, CD30 etc.
Sarcoma	Vimentin, actin, desmin, S100, c-kit etc

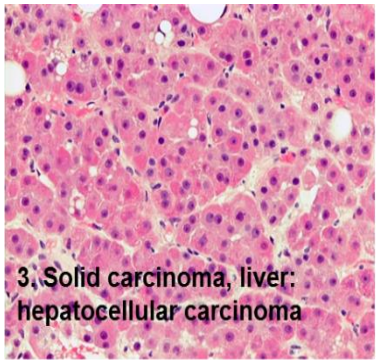
Classification of cancer including CUP: A stepwise pathological approach

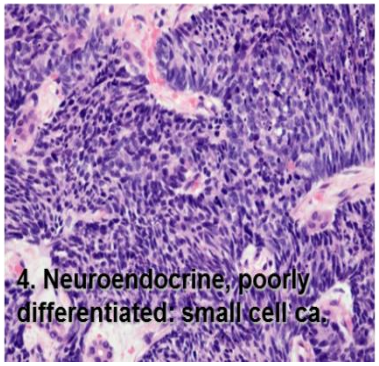
Step 2: if carcinoma or related, identify subtype

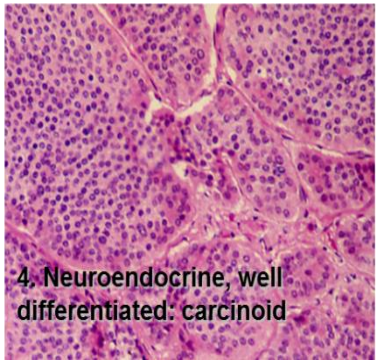
- Adenocarcinoma
- Squamous ca.
  - Transitional ca.
- Solid organ ca. (hepatocellular, renal, thyroid, adrenal)
- Neuroendocrine ca.
- (Germ cell tumour)
- (Mesothelioma)

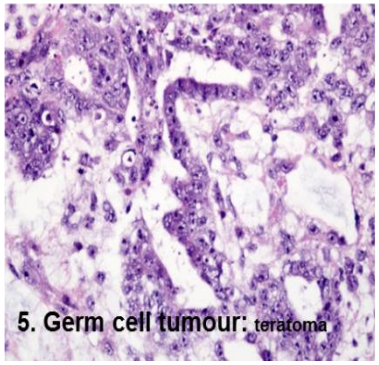












Immunohistochemistry for CUP: Step 2: if carcinoma, identify subtype

Adenocarcinoma	**CK7, CK20, PSA** and other adenoca markers
Squamous ca	**CK5, p63**
Transitional ca	CK7, CK20, uroplakin , GATA3
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

Expression of cytokeratins 7 and 20 in carcinomas and related tumours

	CK7 positive	CK7 negative
CK20 positive	**Gastro-intestinal adenocarcinomas and transitional cell carcinoma** Pancreas & biliary tract (one-third) Stomach (one-quarter) Ovary (mucinous: but many of these likely to be metastatic from gut) Transitional cell carcinoma (two-thirds)	**Gastro-intestinal adenocarcinomas** Colorectum Stomach (one-third) Neuroendocrine tumor of Merkel cell type (poorly differentiated)
CK20 negative	**Many adenocarcinomas** Breast Lung (adenocarcinoma) Ovary (serous & endometrioid) Pancreas & biliary tract (two-thirds) Stomach (one-sixth) Endometrium Salivary tumors Thyroid tumors Transitional cell carcinoma (one-third) Neuroendocrine, poorly differentiated: small cell carcinoma (one-quarter) Malignant mesothelioma (two-thirds)	**Prostatic and other adenocarcinomas plus solid organ, squamous and most neuroendocrine carcinomas** Prostate Stomach (one-sixth) Squamous carcinoma Germ cell tumor Hepatocellular carcinoma Renal (clear) cell carcinoma Adrenocortical carcinoma Neuroendocrine, poorly differentiated: small cell carcinoma (three-quarters) Malignant mesothelioma (one-third)

Classification of cancer including CUP: A stepwise pathological approach

Step 3: if adeno-carcinoma, predict possible primary sites

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

	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

IHC markers commonly used for subtyping of carcinomas

	Marker often used:	Comments on sensitivity and specificity
Adenocarcinoma	CK7, CK20, PSA and other adenoca markers	
Squamous ca	CK5, p63, p40	80-90% sensitive for squamous and basal carcinomas and (p63) for transitional cell carcinomas; also seen in minority of adenocarcinomas especially breast (basal phenotype) thus moderately specific
Transitional ca	p63, CK7, CK20, urothelin, GATA3	
Neuroendocrine ca	Chromogranin, CD56, synaptophysin; TTF1 in some	TTF1 expressed in most poorly differentiated neuroendocrine carcinomas (small cell) and in some well-differentiated neuroendocrine tumours of lung origin (c.f. CDX2 in those of intestinal origin)
Solid ca: renal	RCC, PAX8, Napsin A, luminal membranous CD10	RCC 55-86% sensitive
Solid ca: liver	Hepar1, canalicular CD10, glypican-3	Hepar1 55-99% sensitive; moderately specific (may stain some adenocarcinomas)
Solid ca: thyroid	TTF1, thyroglobulin, PAX8	
Solid ca: adrenal	Melan-A, inhibin	50-100% sensitive
(Germ cell tumour)	OCT4, PLAP, HCG, AFP, glypican-3	OCT4 nearly 100% sensitive and 100% specific for embryonal carcinoma and seminoma; PLAP highly sensitive and moderately specific; AFP yolk sac tumour; HCG choriocarcinoma
(Mesothelioma)	Calretinin, CK5, CK7, D2-40, WT1	(BerEP4 and ERA negative)

Biomarker challenges

- Classic biomarkers for primary site are highly specific (at top of ranking and decision tree)
- Specific biomarkers for stomach & pancreas are lacking

Marker	Tumours of	% Sensitivity	% Specificity
PSA	Prostate	100	**99**
TTF1	Lung	91	**98**
CDX2	Colon	83	**96**
CDX2	Colon and stomach	56	**98**
CK20	Colon	68	**91**
CK20	Colon, stomach and pancreas	36	**97**
GCDFP-15	Breast	54	**96**
ER	Breast and ovary	74	**95**
CA125	Ovary and pancreas	88	**88**
Mesothelin	Ovary and pancreas	85	**85**
Lysozyme	Stomach and pancreas	65	**69**
CK7	(Stomach and pancreas) versus colon	72	**96**

CUP: new IHC reviews

Practical Applications in Immunohistochemistry

Carcinomas of Unknown Primary Site

Patricia L. Kandalaft, MD; Allen M. Gown, MD

• *Context.*—**Identification of the site of origin of carcinoma of unknown primary using immunohistochemistry is a frequent requirement of anatomic pathologists. Diagnostic accuracy is crucial, particularly in the current era of targeted therapies and smaller sample sizes.**

Objectives.—**To provide practical guidance and suggestions for classifying carcinoma of unknown primary using both proven and new antibodies, as well as targeting panels based on integration of morphologic and clinical features.**

Data Sources.—**Literature review, the authors' practice experience, and authors' research.**

Conclusions.—**With well-performed and interpreted immunohistochemistry panels, anatomic pathologists can successfully identify the site of origin of carcinoma of unknown primary. It is crucial to understand not only the diagnostic uses of the many available antibodies but also the potential limits and pitfalls.**

(*Arch Pathol Lab Med.* **2016;140:508–523; doi: 10.5858/arpa.2015-0173-CP)**

It is estimated that approximately 4% of all patients with cancer present with carcinomas of unknown primary (CUPs), representing a higher incidence than known malignancies such as non-Hodgkin lymphoma or ovarian cancer.[1] The identification of a primary site in such a setting has taken on dramatically increased clinical relevance, given the differences in prognosis and treatment, particularly targeted therapies of carcinomas of various primary sites. By integrating morphology with well-performed and well-interpreted immunohistochemistry (IHC), the pathologist can frequently provide definitive diagnostic information in most cases regarding the most likely primary site or sites of the carcinoma presenting as metastases. With the ongoing additions of lineage-specific transcription factors, pathologists have available an increasing number of relatively inexpensive IHC "tools," which more accurately identify CUP. In this era of health care cost containment, and the need to provide clinicians with a relatively quick diagnosis, IHC remains the gold standard at diagnosing CUP. There have been a number of recent publications advocating for the use of gene expression–based tests in the setting of CUP.[2–4] Both methodologies offer a similar range of accuracy in tumor classification (ranging from around 75% and greater); however, in our practice, gene expression–based tests are rarely used or required. Although the proposed algorithm of using gene expression profiling when the initial round of IHC panel is inconclusive may be a useful complement to IHC in some laboratories, in our practice, we often include an additional round of carefully selected and targeted IHC stains in such a scenario, which frequently leads to a diagnosis.

In general, there are 2 classes of antibody markers that can be of assistance in the workup of CUP: (A) antibodies to keratins, and (B) antibodies to organ-restricted markers.

KERATINS

Low–Molecular-Weight Keratins Versus High–Molecular-Weight Keratins

Keratins, previously referred to as *cytokeratins*, have recently undergone a change in nomenclature to accommodate the sequencing of the human genome and discovery of several novel keratin genes.[5] The somewhat arbitrary division of the keratin universe into "high– versus low–" molecular-weight keratins corresponds to certain aspects of the tissue distribution of keratins. Thus, low–molecular-weight keratins (eg, keratin [K] 8, K18) are expressed by "simple" epithelium, such as glandular epithelium of the gastrointestinal (GI) tract, hepatocytes, among others, and high–molecular-weight keratins (eg, K5, K14, K17) are expressed by "complex" epithelium, such as stratified (squamous, transitional) epithelium, as well as ductal and

Antibodies to:	Localization of Signal	Sensitivity	Specificity	
Estrogen receptors	Nuclear	Moderate	Moderate	Endo ad ov
SCDFP-15	Cytoplasmic	Low	Moderate	Saliv gl
Mammaglobin	Cytoplasmic	Low	Moderate	Saliv gl
GATA3	Nuclear	High	Moderate	Saliv tra sk
Villin	Membranous brush border	High	Moderate	Subs ca ar
CDX2	Nuclear	High	High	Subs ga
HepPar1	Cytoplasmic	Moderate	High	Hep ac
Arginase	Nuclear and cytoplasmic	High	High	Hep
TTF-1	Nuclear	High	High	Neu ot
Napsin A	Cytoplasmic	High	High	GYN su ar
PAX8	Nuclear	Very high	Moderate	Thyr C
WT1	Nuclear	Very high	High	Mes
Prostate-specific antigen	Cytoplasmic	Very high	Very high	
NKX3.1	Nuclear	Very high	Very high	
PAX8	Nuclear	Moderate	Moderate	GYN
p63	Nuclear	Very high	Very high	Thyr tu ne tro
CK20	Nuclear	Very high	Very high	Thyr tu tu
Thyroid	Thyroglobulin	Cytoplasmic	High	Very high	...
Thyroid	PAX8	Nuclear	Very high	Moderate	GYN and renal CAs
Transitional cell	Uroplakin	Cell membranous	Low	High	...
Transitional cell	GATA3	Nuclear	High	Moderate	Breast cancers, salivary gland CAs, skin adnexal tumors

Abbreviations: CA, carcinoma; GI, gastrointestinal; GYN, gynecologic; NE, neuroendocrine.

Accepted for publication September 4, 2015.
Published as an Early Online Release October 12, 2015.

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The authors have no relevant financial interest in the products or companies described in this article.

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Metastatic Carcinoma of Unknown Primary: Diagnostic Approach Using Immunohistochemistry

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Abstract: Carcinoma of unknown primary origin (CUP) is one of the 10 most prevalent malignancies. CUP patients in whom a site of origin can be ascribed have better outcomes than those in which the primary tumor remains unidentified. Among the tools available to pathologists in approaching these lesions, immunohistochemistry is a reliable, inexpensive, and widely available resource. New markers continue to emerge, which, in combination with other historically useful antibodies, allow rapid and accurate identification of primary site in an increasing number of cases. This review discusses the approach to the diagnosis of CUP using immunohistochemistry and outlines some of the most useful markers with a particular focus on the utility of lineage-restricted transcription factors, including CDX2, NKX3-1, PAX8, SATB2, TTF-1, and SF1.

Key Words: carcinoma of unknown primary, metastasis, immunohistochemistry, transcription factors, tumor biomarkers

(*Adv Anat Pathol* 2015;22:149–167)

Carcinoma of unknown primary origin (CUP) is defined by histologically confirmed metastatic carcinoma in the absence of clinical, radiographic, or pathologic identification of a primary site. Approximately 3% to 5% of new malignant diagnoses are classified as CUP. As such, CUP ranks among the 10 most prevalent forms of cancer in both males and females.[1] The most common sites of involvement include lymph nodes, liver, bones, and lungs.[2] A review of 12 autopsy series in patients with CUP showed that a primary site was identified at autopsy in 73% of cases. The most common sites of origin included pancreaticobiliary tract, lung, and kidney.[3]

Overall, patients with CUP have a poor prognosis, with median survival times of between 8 and 11 months. Clinically, approximately 15% of CUP cases can be classified into categories that predict primary site, and accordingly allow for directed therapy. Examples include female patients with axillary lymph node involvement (presumed breast primary) and squamous cell carcinoma involving cervical lymph nodes (presumed head and neck primary). These patients have improved outcomes compared with the 85% of patients in whom no primary site can be presumed and therefore no site-specific treatment can be offered.[4] As more targeted therapies emerge, the survival benefit of assigning primary site in newly diagnosed metastatic carcinomas will continue to increase. Therefore, clinicians, radiologists, and pathologists must take an active role in identifying sites of origin in metastatic carcinoma.

Immunohistochemistry has an essential role in the evaluation of biopsies and fine-needle aspirates of metastatic tumors. It has a relatively low cost compared with other techniques such as advanced imaging studies and molecular genetic analysis, and it can be performed easily on paraffin-embedded tissue, even when only scant malignant cells are present. A wide array of new immunohistochemical markers has emerged in the last decade, most notably antibodies directed against lineage-specific transcription factors, which have improved specificity for primary site determination compared with antibodies that recognize keratins and other cytoplasmic and membranous antigens. Here we review the immunohistochemical approach to biopsies of CUP with a particular focus on the application of lineage-restricted markers in this context.

KERATIN FAMILY MEMBERS IN CARCINOMA

Keratins, a family of intermediate filament proteins expressed in epithelial cells, have historically been useful in confirming epithelial origin in poorly differentiated malignancies,[5] although other tumor types, including mesothelioma[6] and some sarcomas such as synovial sarcoma[7] also express keratins. There are 54 functional keratin genes in the human genome.[8] Although the patterns are not entirely specific, differential expression of the protein products from these genes in epithelial cells from various anatomic sites can be exploited to suggest possible primary sites in the setting of metastatic CUP.

Antibodies directed against low–molecular-weight keratins, such as CK8 (including clone CAM5.2[9]) and CK18, react with most glandular epithelial cells as well as hepatocytes. In contrast, antibodies specific for high–molecular-weight keratins, such as CK5 and CK14 (including clone 34βE12, which recognizes CK1, CK5, CK10, and CK14[10]), react predominantly with squamous epithelium and urothelium. Accordingly, antibodies directed against low–molecular-weight keratins are useful to support a diagnosis of adenocarcinoma and hepatocellular carcinoma (HCC), whereas those directed against high–molecular-weight keratins are helpful to confirm the diagnosis of squamous cell carcinoma and urothelial carcinoma.

Among keratin family members, CK7 (KRT7) and CK20 (KRT20) have been most widely used to predict primary site.[11] The most common CK7 and CK20 profiles are shown in Table 1. Although these expression patterns may be useful to prioritize one site of origin over another and to direct further workup, cases that do not fit these profiles are encountered frequently. Furthermore, the CK7-positive, CK20-negative immunophenotype is most common in CUP; this profile is not particularly helpful to suggest a specific anatomic site of origin. CK7 and CK20

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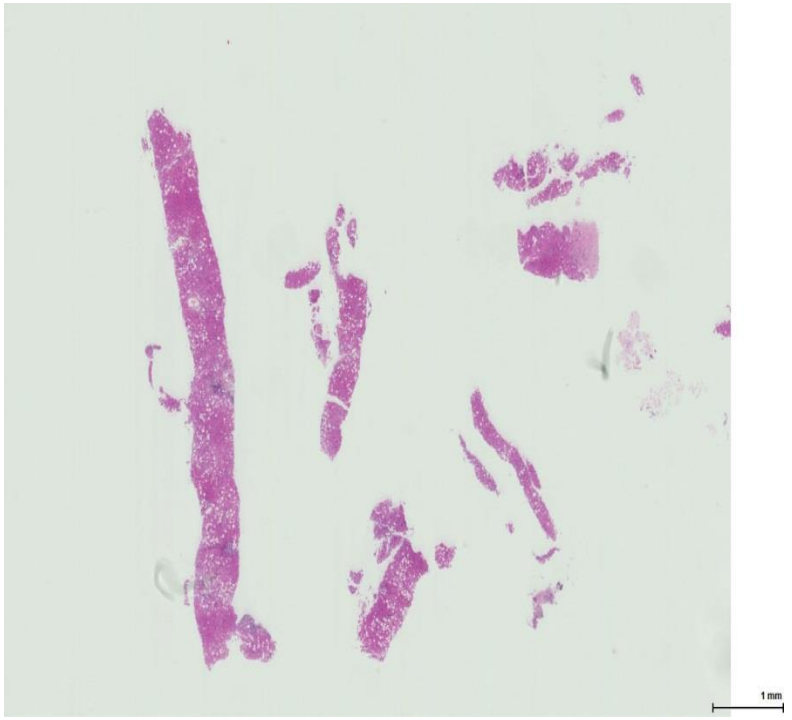
Selection of further or newer biomarkers for carcinoma type and site

Biomarker	Carcinoma subtype	Also identifies
GATA3	Breast carcinoma, transitional cell carcinoma	Salivary gland, skin adnexal tumours
(Villin)	Colorectal	Some renal, lung, ovarian and endometrial carcinomas
SATB2	Colorectal (c.f. not in other gastro-intestinal neoplasms)	Some renal cell carcinomas, osteosarcomas
Arginase	Hepatocellular carcinoma	
Napsin A	Lung adenocarcinoma, renal cell carcinoma	Gynaecological clear cell carcinoma
WT1	Ovarian serous	Mesothelioma
PAX8	Ovarian serous, endometrioid, clear cell and mucinous; renal cell carcinoma	Thyroid
p40	Squamous and transitional cell carcinoma	Salivary gland tumours, thymoma & trophoblastic tumours
Steroidogenic Factor 1	Adrenocortical neoplasms (c.f. other clear cell neoplasms)	

Diagnostic difficulties: not enough tissue



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

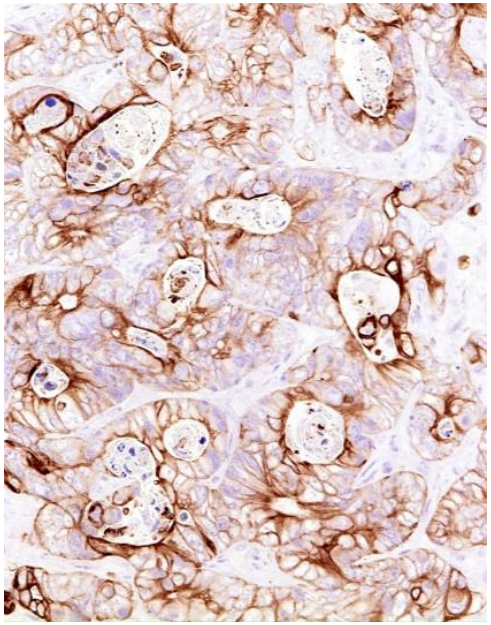
Diagnostic difficulties: difficult morphology

- Common diagnostic difficulties in classical tumour typing for CUP are with:
  - “Very poorly differentiated or undifferentiated tumours
    - Adenocarcinoma, even well-differentiated, without obvious primary site
      - Pancreatico-biliary including cholangiocarcinoma
      - Gastro-oesophageal
      - Ovarian mucinous
      - Atypical tumours from lung, breast etc

Diagnostic difficulties: difficult immunohistochemistry

- 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 (study showed 7-8 was usual in CUP)
- In CUP, a barrier to correct tumour classification is not using the most appropriate markers

Can we quantify performance of current pathology in CUP classification?



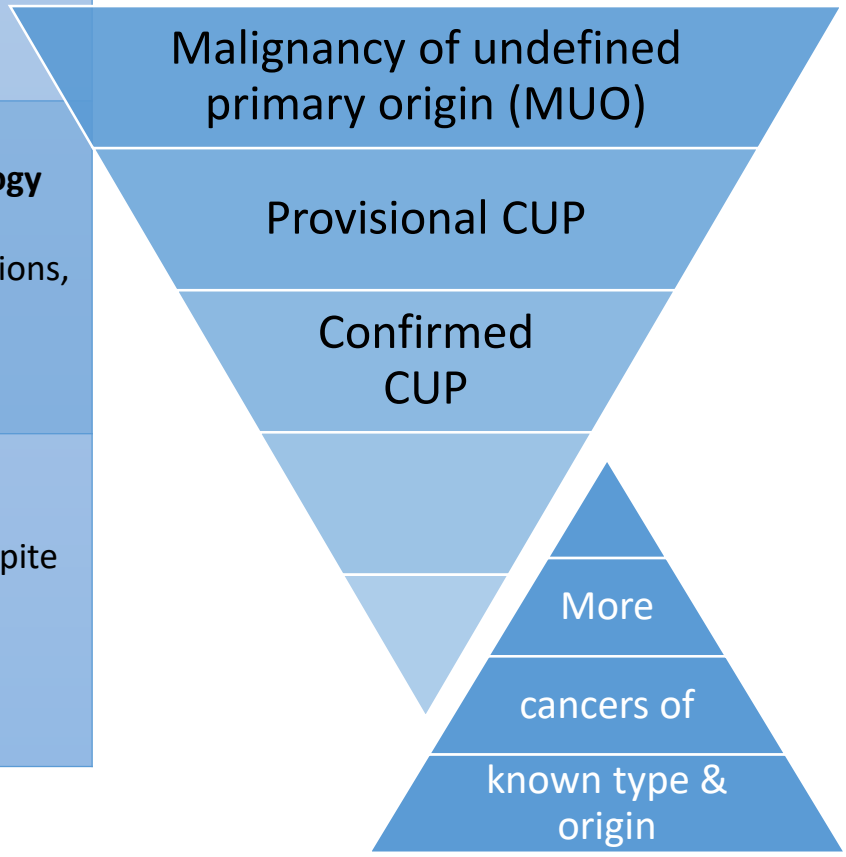
- Only 5-6 studies identified in recent meta-analysis
- **Sensitivity of IHC panels for primary site was consistent, around 82% in mixed primary and metastatic tumours and 66-70% in metastases alone**
- Confirms metastases are harder to classify than primary tumours by IHC and sets baseline for comparison with molecular tests

NICE guidance on metastatic malignant disease of unknown primary origin

Malignancy of undefined primary origin (MUO)	Metastatic malignancy identified on the basis of a limited number of tests, without an obvious primary site, before comprehensive investigation
Provisional carcinoma of unknown primary origin (provisional CUP)	Metastatic epithelial or neuroendocrine malignancy identified on the basis of histology or cytology, with no primary site detected despite a selected initial screen of investigations, before specialist review and possible further specialised investigations
Confirmed carcinoma of unknown primary origin (confirmed CUP)	Metastatic epithelial or neuroendocrine malignancy identified on the basis of final histology, with no primary site detected despite a selected initial screen of investigations, specialist review, and further specialised investigations as appropriate.

CUP definitions with MDT approach

Malignancy of undefined primary origin (MUO)	**Metastatic malignancy** identified on the basis of a limited number of tests, **without an obvious primary site, before comprehensive investigation.**
Provisional carcinoma of unknown primary origin (**provisional CUP**)	**Metastatic epithelial or neuro-endocrine malignancy identified on the basis of histology or cytology, with no primary site detected** despite a **selected initial screen** of investigations, before specialist review and possible further specialised investigations.
Confirmed carcinoma of unknown primary origin (**confirmed CUP**)	**Metastatic epithelial or neuro-endocrine malignancy identified on the basis of final histology, with no primary site detected** despite a selected initial screen of investigations, **specialist review, and further specialised investigations** as appropriate.

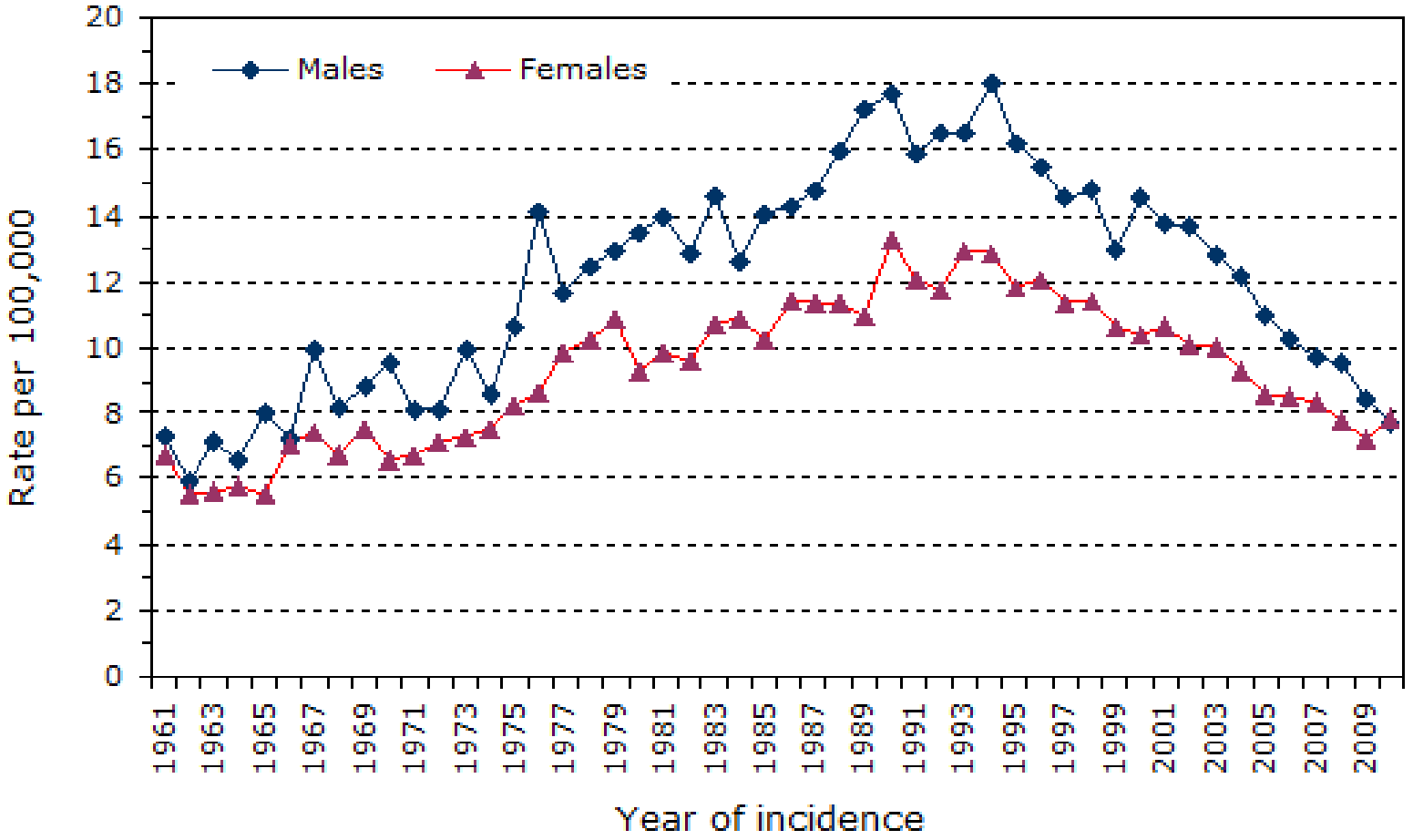


CUP patient pathway with CUP team & MDT

- NICE guidelines mandated CUP networks across hospitals
  - CUP team in each hospital with cancer care
    - Link with acute oncology
  - CUP specialist nurse or keyworker
    - Major role in coordinating patient's care
  - CUP network **multidisciplinary team (MDT) including pathologist** set up to review treatment and care of patients with confirmed CUP
  - Provide clinical management & support like type- and site-specific cancer MDTs

- Avoid “MDT tennis”

With these approaches, CUP incidence, which had increased, is now decreasing



CUP teams managing residual “unfavourable” or “poor prognosis” CUP

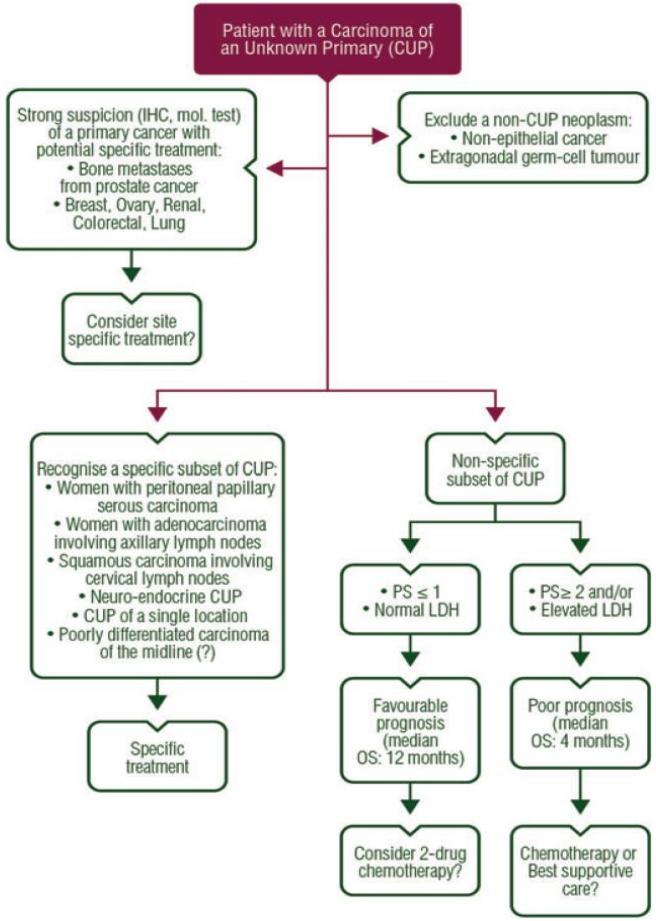


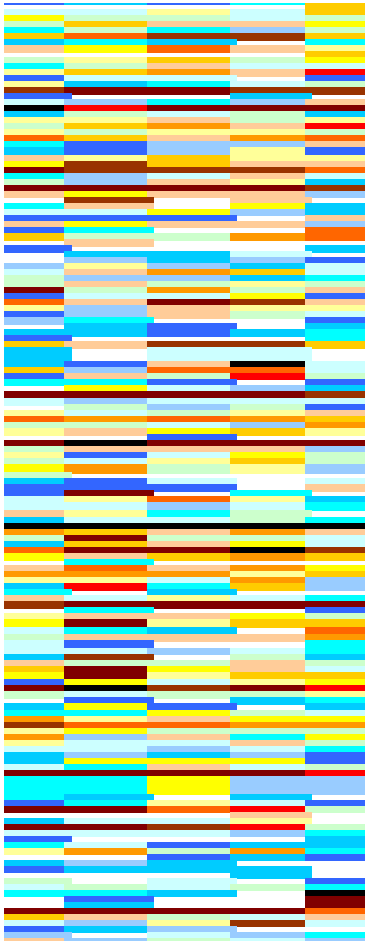
Figure 2. Clinical management of patients presenting with CUPs. IHC,

Unfavourable subset

- Adenocarcinoma metastatic to the liver or other organs
- Non-papillary malignant ascites (adenocarcinoma)
- Multiple cerebral metastases (adenocarcinoma or squamous carcinoma)
- Several lung or pleural metastases (adenocarcinoma)
- Multiple metastatic lytic bone disease (adenocarcinoma)
- Squamous-cell carcinoma of the abdominopelvic cavity

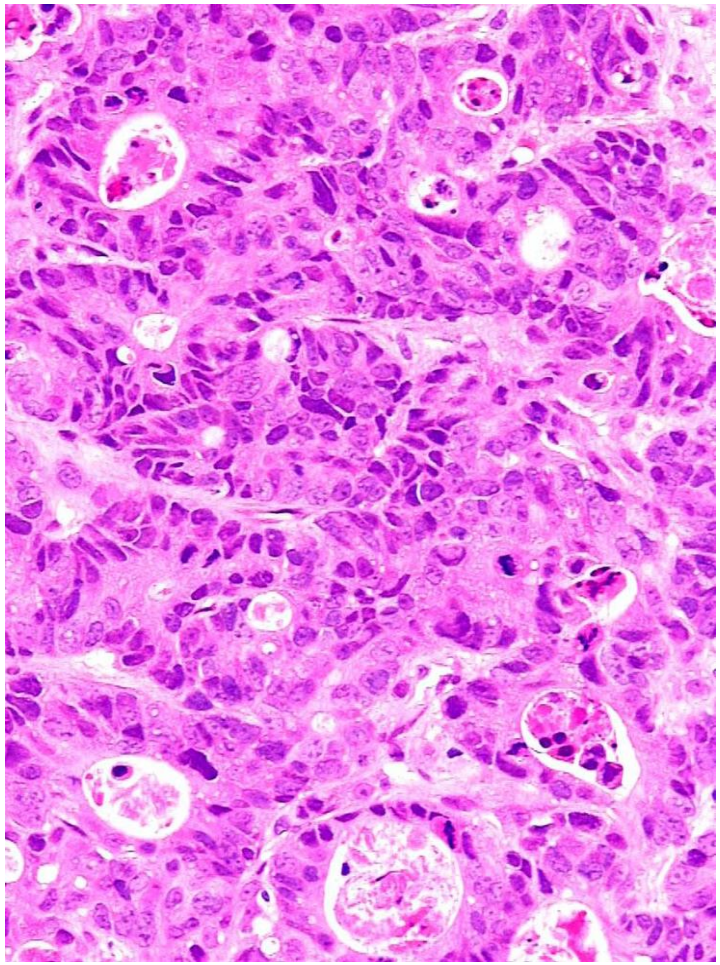
- Residual “poor prognosis” CUP pathology is mainly:
  - Poorly differentiated tumour, or
  - Adenocarcinoma without obvious primary site, often positive for CK7 but not much else

Molecular profiling for primary site: rationale



- "Different tissue types have distinct RNA profiles" (…or protein, or DNA…)
- Three RNA tests commercially available:
  - Tissue of Origin (TOO) test (Response Genetics)
  - Cancer Type ID (CTID) (bioTheranostics)
  - Cancer Origin (miRview mets2) (Rosetta Genomics)
- Yield tumour type, site and/or subtype i.e. classic taxonomy
- IHC and mRNA molecular profiling use similar tissue-specific genes (e.g. PSA etc):
  - Molecular profiling tests many more genes and may be less subjective

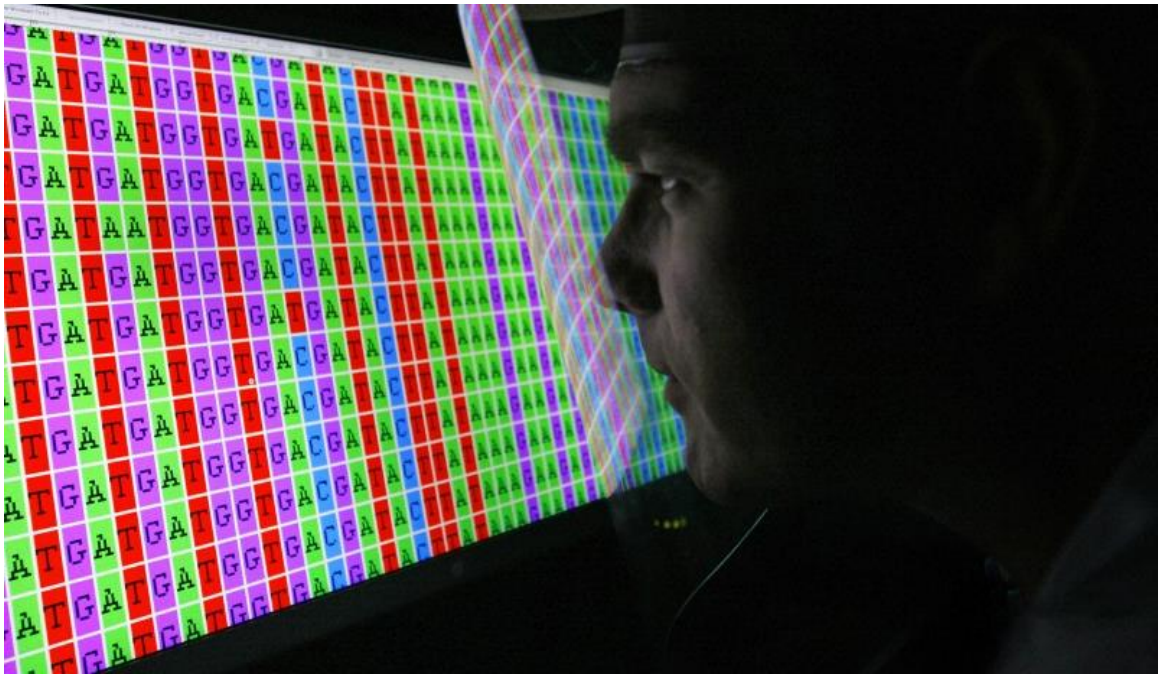
Molecular profiling for primary site in practice: limits



- Tumours difficult to diagnose using morphology and IHC are often also difficult for molecular profiling
- Overall around 10% of tests fail i.e. yield no result
- All tests may find difficult:
  - Limited or necrotic tissue
  - Poorly differentiated tumours
  - Pancreatic, gastro-intestinal and lung cancers

Molecular analysis for actionable mutations

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



US NCCN Clinical Practice Guidelines on Occult Primary

Recommendations 2014	• Although GEP (Gene Expression Profiling) has a diagnostic benefit, clinical benefit has not been demonstrated. The panel recommends against GEP as standard management, although 20% of the panel believes the diagnostic benefit of GEP warrants its routine use...
Recommendations 2015	• Until more robust outcomes and comparative effectiveness data are available, pathologists and oncologists must collaborate on the judicious use of these modalities (IHC and GEP) on a case-by-case basis, with the best possible individualized patient outcome in mind...

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

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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

Appendix B Histopathology reporting proforma for Cancer of Unknown Primary (CUP)

Surname..... Forenames..... Date of birth..... Sex.....
Hospital..... Hospital no.....
NHS/CHI no.....
Date of receipt..... Date of reporting..... Report no..... Pathologist.....
Surgeon.....

Site of sample – circle:

liver / lung / brain / lymph node (specify site .....)

bone (specify site.....) / other (specify site .....)

Type of sample – circle:

Small biopsy e.g. needle core / small excision biopsy / effusion cytology / FNA

other (specify.....)

Morphology – circle all that apply:

Epithelioid / sarcomatoid or spindle / "small blue cell" / undifferentiated or pleomorphic

Other (specify.....)

Immunohistochemistry – list markers employed:

Positive.....

Equivocal.....

Negative.....

Have you excluded lymphoma? YES/ NO **Have you excluded germ cell tumour? YES/NO**

Consultant double reporting? YES/NO **Expert second opinion? YES/NO**

If no expert second opinion sought, state reason.....

Confirmation of discussion CUP MDT: YES/NO Date of discussion at CUP MDT.....

Summary of blood cancer markers.....

Summary of imaging modalities.....

Following integration of all test modalities, confirmed CUP status achieved clinically: YES/NO

Comment:.....

Reporting pathologist 1..... Reporting pathologist 2.....

SNOMED codes T..... M80006

Optional non-core data items

Actionable mutations e.g. KRAS, ALK, EGFR etc : YES/NO

Positive……

Negative……

…

Molecular markers including mRNA profiling (if undertaken):

Result:……

Summary of CUP Past, Present, Future
Classification of CUP through the Decades