CANCER OF UNKNOWN PRIMARY
A Complex Disease

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DEAN, MEDICAL SCHOOL, UNIVERSITY OF CYPRUS

ESO / ESMO MASTERCLASS,
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WHAT IS CANCER OF UNKNOWN PRIMARY (CUP)?

- Is a clinical disorder where patients present with histologically confirmed metastatic cancer for which standard diagnostic investigations failed to identify the primary site.
- Median age is 65 years, more common in men.
- It accounts for 3-5% of all human cancers.
- It is considered to be the 7th-8th most frequent malignant tumor.
CLINICAL CHARACTERISTICS AT PRESENTATION

- Early dissemination
- Clinical absence of primary
- Aggressiveness
- Unpredictable metastatic pattern i.e. hidden pancreatic cancer has higher incidence of bone or lung metastases
Cancer of Unknown Primary Site:

One or more Diseases?
# Histo logical Classification

<table>
<thead>
<tr>
<th><strong>Histology</strong></th>
<th><strong>Incidence</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma</td>
<td></td>
</tr>
<tr>
<td>Well to moderately differentiated</td>
<td>50 %</td>
</tr>
<tr>
<td>Poorly or undifferentiated</td>
<td>35 %</td>
</tr>
<tr>
<td>Squamous cell carcinoma</td>
<td>10 %</td>
</tr>
<tr>
<td>Undifferentiated neoplasms</td>
<td>5 %</td>
</tr>
<tr>
<td>Not specified carcinoma</td>
<td></td>
</tr>
<tr>
<td>Neuroendocrine tumors</td>
<td></td>
</tr>
<tr>
<td>Lymphomas</td>
<td></td>
</tr>
<tr>
<td>Germ cell tumors</td>
<td></td>
</tr>
<tr>
<td>Melanomas</td>
<td></td>
</tr>
<tr>
<td>Sarcomas</td>
<td></td>
</tr>
<tr>
<td>Embryonal malignancies</td>
<td></td>
</tr>
</tbody>
</table>
# Clinicopathological Entities of CUP

<table>
<thead>
<tr>
<th>Organ</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver (mainly)</td>
<td>AdenoCa M or P diff</td>
</tr>
<tr>
<td>and/or other organs</td>
<td></td>
</tr>
<tr>
<td>Lymph nodes</td>
<td>U or P diff Ca</td>
</tr>
<tr>
<td>Mediastinal – Retroperitoneal (midline distribution)</td>
<td></td>
</tr>
<tr>
<td>Axillary</td>
<td>AdenoCa W to P diff</td>
</tr>
<tr>
<td>Cervical</td>
<td>SCC Ca</td>
</tr>
<tr>
<td>Inguinal</td>
<td>U Ca, SCC, mixed SCC / adenoCa</td>
</tr>
</tbody>
</table>

*W = well,  M = moderately,  P = poorly,  U = undifferentiated*
Peritoneal cavity

Peritoneal adenocarcinomatosis in females

Malignant ascites of other unknown origin

Papillary or serous adenocarcinoma
(± psammoma bodies)

Mucin adenocarcinoma M or P diff
(± signet ring cells)

Lungs

Pulmonary metastases

Pleural effusion

Adenocarcinoma various diff
Adenocarcinoma M or P diff

W = well, M = moderately, P = poorly, U = undifferentiated
**Bones**
(solitary or multiple) AdenoCa of various diff

**Brain**
(solitary of multiple) AdenoCa of various diff or squamous cell Ca

**Neuroendocrine tumors**
P diff Ca with neuroendocrine features (mainly), low-grade neuroendocrine Ca, small cell anaplastic Ca

**Melanoma**
U neoplasm with melanoma features.

$W = well, \quad M = moderately, \quad P = poorly, \quad U = undifferentiated$
WHAT IS THE OPTIMAL INVESTIGATIONAL DIAGNOSTIC APPROACH FOR THE IDENTIFICATION OF THE PRIMARY TUMOR?
HOW DO WE SEARCH FOR THE PRIMARY?

By HISTOPATHOLOGY
- Immunohistochemistry
- Advanced Molecular Technology

By IMAGING
- Conventional Radiology
- Ultrasonography
- CT-scans
- MRIs
- PET-scans
- Mammography

By ENDOSCOPY
- ENT panendoscopy
- Bronchoscopy
- Colonoscopy
- Proctoscopy
- Colposcopy
By

HISTOPATHOLOGY
STEPS OF IMMUNOHISTOCHEMICAL DIAGNOSTIC APPROACH FOR CUP

STEP 1  (Detects broad type of cancer)

- Carcinoma: AE 1/3 pancytokeratin
- Lymphoma: Common leucocyte antigen (CLA)
- Melanoma: S100, HMB45
- Sarcoma: S100, Vimentin
<table>
<thead>
<tr>
<th>Tumour Type</th>
<th>Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma</td>
<td>CK 7/20, PSA</td>
</tr>
<tr>
<td>Germ cell tumour</td>
<td>PLAP, OCT4, AFP, HCG</td>
</tr>
<tr>
<td>Hepatocellular Carcinoma</td>
<td>Hepar 1, canalicular</td>
</tr>
<tr>
<td></td>
<td>pCEA/CD10/CD13</td>
</tr>
<tr>
<td>Renal cell carcinoma</td>
<td>RCC, CD10</td>
</tr>
<tr>
<td>Thyroid carcinoma</td>
<td>TTF1, thyroglobulin</td>
</tr>
<tr>
<td>Neuroendocrine carcinoma</td>
<td>Chromogranin, synaptophysin,</td>
</tr>
<tr>
<td></td>
<td>PGP 9.5, CD56</td>
</tr>
<tr>
<td>Squamous carcinoma</td>
<td>CK 5/6, p63</td>
</tr>
<tr>
<td>Tissue</td>
<td>Markers</td>
</tr>
<tr>
<td>-----------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Prostate</td>
<td>PSA, PAP</td>
</tr>
<tr>
<td>Lung</td>
<td>TTF1</td>
</tr>
<tr>
<td>Breast</td>
<td>GCDFP-15, mammaglobulin, ER</td>
</tr>
<tr>
<td>Colon</td>
<td>CD X 2, CK 20</td>
</tr>
<tr>
<td>Pancreas/Biliary</td>
<td>CD X 2, CK 20, CK7</td>
</tr>
<tr>
<td>Ovary</td>
<td>ER, Ca 125, mesothelin</td>
</tr>
</tbody>
</table>
Cytokeratins (CKS)

Monoclonal antibodies against cytokeratin polypeptides CK7 and CK20
CK7  CK20

CK7 + CK20 +
- Urothelial tumors
- Ovarian mucinous adenocarcinoma
- Pancreatic adenocarcinoma
- Cholangiocarcinoma

CK7 + CK20 -
- Lung adenocarcinoma
- Breast carcinoma
- Thyroid carcinoma
- Endometrial carcinoma
- Cervical carcinoma
- Salivary gland carcinoma
- Cholangiocarcinoma
- Pancreatic carcinoma

CK7 - CK20 +
- Colorectal Carcinoma
- Merkel cell carcinoma

CK7 - CK20 -
- Hepatocellular carcinoma
- Renal cell carcinoma
- Prostate carcinoma
- Squamous cell & small cell lung carcinoma
- Head & neck carcinoma
MOLECULAR ANALYSIS [Microarray Platforms]

> 80 - 90 % accuracy
Gene expression profiling

<table>
<thead>
<tr>
<th>Assay</th>
<th>Platform</th>
<th>Tissue</th>
<th>No. of Tumor types</th>
<th>Number of genes</th>
<th>Accuracy in known tumors (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veridex</td>
<td>RT-PCR mRNA</td>
<td>FFPE</td>
<td>6 and ”other”</td>
<td>10</td>
<td>76</td>
</tr>
<tr>
<td>Pathwork Diagnostics</td>
<td>cDNA microarray</td>
<td>Frozen/FFPE</td>
<td>15</td>
<td>1500</td>
<td>89</td>
</tr>
<tr>
<td>Tissue of Origin test</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rosetta Genomics</td>
<td>RT-PCR miRNA</td>
<td>FFPE</td>
<td>22</td>
<td>48 miRNAs</td>
<td>86</td>
</tr>
<tr>
<td>MiReview met</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>bioTheranostics</td>
<td>RT-PCR mRNA</td>
<td>FFPE</td>
<td>39 (including subtypes)</td>
<td>92</td>
<td>86</td>
</tr>
<tr>
<td>CancerType ID</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**ENDOSCOPY**

- Should always be symptoms - or sings oriented investigational procedures

**SERUM TUMOR MARKERS**

- In general, routine evaluation of current commonly used markers have not been proven of any prognostic or diagnostic assistance
WHAT IS THE OPTIMAL THERAPEUTIC APPROACH OF CANCER OF UNKNOWN PRIMARY?
DIAGNOSTIC AND THERAPEUTIC MANAGEMENT OF CANCER OF AN UNKNOWN PRIMARY

N. Pavlidis, E. Briasoulis, J. Hainsworth, E.A. Greco
CUP

FAVOURABLE OR GOOD PROGNOSIS SUBSETS

UNFAVOURABLE OR POOR PROGNOSIS SUBSETS
DO WE HAVE EFFECTIVE DRUGS FOR CANCER OF UNKNOWN PRIMARY

OR

WE JUST HAVE RESPONSIVE SUBSETS OF PATIENTS?
THE FAVOURABLE SUBSETS
OR
GOOD PROGNOSIS SUBSETS
Favourable Subsets

1. Women with adenocarcinoma involving only axillary lymph nodes.

2. Women with papillary adenocarcinoma of peritoneal cavity.

3. Squamous cell carcinoma involving cervical lymph nodes

4. Poorly differentiated neuroendocrine carcinomas. Merkel cell carcinoma of unknown primary (localized disease)

5. Adenocarcinoma with a colon-profile (CK 20+, CK 7-, CDX 2+)

6. Men with blastic bone metastases and elevated PSA (adenocarcinoma).

7. Isolated inguinal adenopathy (squamous carcinoma).

8. Patients with a single, small, potentially resectable tumor.
Subset 1

Women with adenocarcinoma involving axillary nodes

- Mostly ductal adenocarcinoma (40% positive ER/PR)
- Mean age 52 years
- Should be managed as stage II breast cancers (axillary dissection, ipsilateral breast radiotherapy, adjuvant chemo/hormone therapy)
- 5-year survival: 72%
Subset 2

Women with primary papillary adenocarcinoma peritoneal cavity

- Similar presentation with advanced ovarian cancer.
- Median age 60 years
- Histopathology: serous or papillary adenocarcinoma
- Serum CA 125 is frequently increased
- Should be treated as stage III-IV ovarian cancer (cytoreduction, followed by platinum/taxanes)
- Responses: 80% (CR: 30-40%), Median survival: 36 months
Subset 3

Squamous cell carcinoma involving cervical nodes

- It constitutes 5% of all head-neck cancers
- Median age 60 years
- Sensitivity of PET-scan to detect the primary is 60%
- Treatment
  - $N_1$ or $N_{2a}$ disease without extracapsular extension: surgery alone
    
    \[
    \text{locoregional control : 80 – 90\%, 5-yr survival : 65\%}
    \]
  - $\geq N_{2b}$ stage or with extracapsular extension: Postoperative chemoradiation
Subset 4

Poorly differentiated neuroendocrine carcinoma

- Frequently affects retroperitoneal, mediastinal, peripheral nodes less frequently liver or bones.
- Should be treated with platinum and/or taxane combinations
- Response: 55% (CR 20%), Survival: 15 months (10-15% long survivors)
Subset 5

Adenocarcinoma with a colon profile (CK20+, CK7-, CDX2+)

- Clinical presentation: abdominal nodes 51%, peritoneum 50%, liver 30%, ascities 27%

- Should be treated as advanced colorectal cancer with chemotherapy / targeting treatment

- Response: 50% (CR 15%, PR 35%)

  Median Survival: 21-37 months
OTHER FAVOURABLE SUBSETS

- Men with blastic bone metastases from an adenocarcinoma and elevated serum PSA ⇒ treat as advanced prostate cancer

- Isolated inguinal adenopathy from squamous cell carcinoma ⇒
  local excision ± radiation

- Patients with a single, small, potentially resectable tumours ⇒
  local excision ± radiation
UNFAVOURABLE SUBSETS

1. Adenocarcinoma metastatic to the liver or other organs
2. Poorly differentiated carcinoma
3. Non-papillary malignant ascites (adenocarcinoma)
4. Multiple cerebral metastases (adenocarcinoma or squamous Ca)
5. Multiple lung/pleural metastases (adenocarcinoma)
6. Multiple metastatic bone disease (adenocarcinoma)
7. Squamous – cell carcinoma of the abdominal cavity
THE UNFAVOURABLE OR POOR PROGNOSIS SUBSETS

- This group of CUP patients represents 80% of cases.
- Usually treated with empirical chemotherapy (i.e. Platinum / Taxanes).
- Responses: 20%
- Median survival: 6-9 months
Prognostication in cancer of unknown primary (CUP): Development of a prognostic algorithm in 311 cases and review of the literature

Dimitrios Petrakis, George Pentheroudakis, Evangelos Voulgaris, Nicholas Pavlidis*
Fig. 1. Overall Survival by CUP Clinicopathologic Subgroups in univariate analysis.
DOES THE IDENTIFICATION OF PRIMARY SITE BY MOLECULAR PROFILING FOLLOWING SITE-SPECIFIC THERAPY IMPROVE PATIENTS’ OUTCOME?

WHAT IS THE EVIDENCE TODAY?
Conclusion

✓ The median survival time of 12.5 months for patients who received assay-directed site-specific therapy compares favorably with previous results using empiric CUP regimens.

✓ Molecular tumor profiling contributes to the management of patients with CUP and should be a part of their standard evaluation.
# SITE SPECIFIC TREATMENTS

<table>
<thead>
<tr>
<th>Predicted Tissue of Origin</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast</td>
<td>Taxane/bevacizumab</td>
</tr>
<tr>
<td>Colorectal</td>
<td>FOLFOX (or variant) + bevacizumab, or FOLFIRI (or variant) + bevacizumab</td>
</tr>
<tr>
<td><strong>Lung cancer, non-small cell</strong></td>
<td>Platinum-based doublet + bevacizumab</td>
</tr>
<tr>
<td>Ovary</td>
<td>Paclitaxel/carboplatin + bevacizumab</td>
</tr>
<tr>
<td>Pancreas</td>
<td>Gemcitabine/erlotinib</td>
</tr>
<tr>
<td>Prostate</td>
<td>Androgen ablation therapy</td>
</tr>
<tr>
<td>Renal</td>
<td>Sunitinib or bevacizumab ± interferon</td>
</tr>
<tr>
<td><strong>Other diagnoses</strong></td>
<td>Standard first-line treatment per guidelines</td>
</tr>
</tbody>
</table>
OVERALL SURVIVAL: Assay-directed treatment vs. empiric treatment

PRESENTED BY: F. Anthony Greco, MD
I respectfully suggest, with regret, that their trial is insufficient to support this claim.

A trial in which patients with CUP are randomly assigned to best empiric therapy versus expression profile-directed therapy, or some similar design, remains needed.
Interpretation We show that the development of a DNA methylation based assay can significantly improve diagnoses of cancer of unknown primary and guide more precise therapies associated with better outcomes. Epigenetic profiling could be a useful approach to unmask the original primary tumour site of cancer of unknown primary cases and a step towards the improvement of the clinical management of these patients.
<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Cases (n)</th>
<th>Specific treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast carcinoma</td>
<td>6</td>
<td>Cyclophosphamide plus doxorubicin plus paclitaxel;</td>
</tr>
<tr>
<td></td>
<td></td>
<td>capecitabine; denosumab; letrozole</td>
</tr>
<tr>
<td>Non-small-cell lung carcinoma</td>
<td>5</td>
<td>Erlotinib; gefitinib; gemcitabine; pemetrexed; vinorelbine</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>4</td>
<td>Gemcitabine plus oxaliplatin; bleomycin; iodised oil; sorafenib</td>
</tr>
<tr>
<td>Ovarian carcinoma</td>
<td>3</td>
<td>Carboplatin plus paclitaxel; doxorubicin</td>
</tr>
<tr>
<td>Endometrial carcinoma</td>
<td>2</td>
<td>Carboplatin plus taxanes; pemetrexed</td>
</tr>
<tr>
<td>Colon carcinoma</td>
<td>2</td>
<td>Fluorouracil plus oxaliplatin plus bevacizumab</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>2</td>
<td>Cisplatin plus gemcitabine; pemetrexed</td>
</tr>
<tr>
<td>Pancreatic carcinoma</td>
<td>2</td>
<td>Paclitaxel; erlotinib; gemcitabine</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>2</td>
<td>Gemcitabine plus docetaxel; ifosfamide; letrozole</td>
</tr>
<tr>
<td>Acute lymphoblastic leukaemia</td>
<td>1</td>
<td>Cyclophosphamide plus doxorubicin plus vincristine plus prednisone</td>
</tr>
<tr>
<td>Prostate carcinoma</td>
<td>1</td>
<td>Sorafenib</td>
</tr>
<tr>
<td>Stomach carcinoma</td>
<td>1</td>
<td>Gemcitabine plus oxaliplatin; capecitabine</td>
</tr>
</tbody>
</table>

EPICUP = microarray DNA methylation signatures.

*Table 4:* Cases of cancer of unknown primary classified by tumour types predicted by EPICUP that received specific therapy (n=31)
Figure: Outcome of patients with cancer of unknown primary who receive a site-specific treatment that matches the EPICUP prediction.
Cancer of unknown primary: time to put the pieces of the puzzle together?

Panagiota Economopoulou, George Pentheroudakis
Authors’ Comments:

• Ideally, a well-designed prospective randomised study could help to identify subsets of patients who would benefit more from targeted therapies, with the view to improve outcomes without maximising costs.

• However, a question that remains unanswered is whether a cancer of unknown primary with a molecular signature of a specific primary behaves similarly to a typical metastatic cancer.
## Ongoing Phase III Randomized Trials with the Use of Molecularly Assigned Therapy

<table>
<thead>
<tr>
<th>Trial</th>
<th>Sponsor</th>
<th>Design</th>
<th>Arms</th>
</tr>
</thead>
<tbody>
<tr>
<td>GEFCAP 104</td>
<td>GEFCAPI, France</td>
<td>RCT Phase III</td>
<td>cDDP + Gemcitabine vs Pathwork test – Based Therapy</td>
</tr>
<tr>
<td>CUP-ONE</td>
<td>CR UK</td>
<td>RCT Phase III</td>
<td>ECX vs Molecularly Assigned Therapy</td>
</tr>
</tbody>
</table>
DO PATIENTS WITH CUP EXPRESS BIOMARKERS USEFUL FOR SELECTING SPECIFIC DRUGS?
Comprehensive tumor profiling identifies numerous biomarkers of drug response in cancers of unknown primary site: analysis of 1806 cases.

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

- 2006 CUP paraffin–embedded specimens (both studies)
- At least one genomic alteration was found in 96% of specimens mainly of adenocarcinoma CUPs, including: ALK, ARAF, BRAF, EGFR, FGR1, 1, KIT, KRAS, MAP2K1, MET, NF1, NF2, NRAS, RAF1, RET, ROS1

CONCLUSION: Comprehensive biomarker profiling of CUP may provide additional choices in the treatment of pts with these difficult to treat malignancies
The Use of Gene Expression Profiling and Mutation Analysis Increases the Cost of Care for Patients With Carcinoma of Unknown Primary; Does it also Improve Survival?

• We must be careful as not all screening tests turn into clinical advances.

• The widespread adoption of novel costly tests without clear evidence that they improve outcomes is not a consequence of enthusiasm for personalized medicine.

• The use of gene profiling should, for the time being, be confined to carefully conducted clinical protocols.

• We favour waiting for the results of an ongoing randomized trial being conducted at Gustave Roussy.

• In the meantime, we believe that there has not yet been any evidence to change the paradigm of care of CUP.
(2016) 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...
**BIOLOGY OF CUP**

SUPPORTERS

CUP doesn’t exist, since 90% of small primaries can be found at autopsy or almost 90% can be identified by GP.

NON-SUPPORTERS

A primary that never grows probably carries a unique i.e. distinct genetic signature, chromosomal instability, etc.

**CHEMOTHERAPY OF CUP**

SUPPORTERS

Data showed responses and survival benefit following specific direct treatment after GP.

NON-SUPPORTERS

Existing information are deriving from non-randomized small studies and from one observational study.

**TARGETED THERAPY OF CUP**

SUPPORTERS

Targeted therapies are effective in some cases.

NON-SUPPORTERS

Only a few anecdotal data exist.
SUPPORTERS

RANDOMIZED TRIALS IN CUP

Randomized prospective studies ARE NOT NEEDED

NON-SUPPORTERS

Randomized prospective studies are URGENTLY NEEDED, especially between specific CUP subsets and relevant known primaries

ENTHUSIASM AND HOPE

We succeeded to make THE UNKNOWN KNOWN
STEP I
Clinical, immunohistochemistry, imaging, endoscopy studies

STEP II
RULE-OUT POTENTIALLY TREATABLE OR CURABLE TUMORS
(i.e. Breast Cancer, Germ-cell Tumors, Lymphomas)

STEP III
CHARACTERIZE THE SPECIFIC CLINICOPATHOLOGICAL ENTITY

TREAT THE PATIENT

FAVOURABLE SUBSETS
[Similarly to relevant primaries with “Curative Intent”]

UNFAVOURABLE SUBSETS
[With empirical chemotherapy with “Palliative Intent” or with specific Rx following gene profiling]