Cancer of Unknown Primary: Diagnosis and Treatment

AND

BIOLOGY

CYE Session
ESMO Conference, Madrid 2014
What we will talk about

• Definition of CUP
• CUP Epidemiology and Statistics
• CUP Pathology and Molecular Assays
• CUP Staging
• CUP Clinicopathologic Subsets
• CUP Prognostication
• CUP Therapy
• CUP Biology
• CUP Clinical Research
Definition of CUP

- Cytologic/pathologic diagnosis of malignancy (systemic metastases) in the absence of an identifiable primary tumour after a standardised diagnostic work up
• Metastases from a primary we simply cannot locate?

• Tumours with not only a primary tissue-specific biology but also with a distinct biological signature, common for most CUPs?
IS THERE EVIDENCE TO SUGGEST THAT CUP HAS PECULIAR AND DISTINCT BIOLOGY COMPARED TO METS FROM KNOWN PRIMARY TUMOURS (KPM)?
PROGNOSIS OF SUB-TYPES OF CUP COMPARED TO METASTATIC CANCER

Bishop JF et al  [New South Wales, Australia]  ASCO 2007 (abs 21010)

- Cancer Registry: 1980 – 2004  
- 65.239 confirmed metastatic cancer patients

46,435 (77%)
Metastatic with known primary

13,280 (22%)
CUP

Median Survival
7 (mos)  
(p<0.0001)  
3 (mos)

Risk of dying by histology

Adenocarcinoma  
80% greater (HR 1.8)

Squamous  
50% less (HR 0.49)

Small cell  
20% less (HR 0.77)

Undifferentiated  
15% less (HR 0.84)
Survival: CUP vs KPM

- Swedish Cancer Registry: 2881 CUP vs 6745 KPM

<table>
<thead>
<tr>
<th>Primary site</th>
<th>N</th>
<th>HR</th>
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</thead>
<tbody>
<tr>
<td>CUP (reference)</td>
<td>2881</td>
<td>1</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>1438</td>
<td>0.61</td>
</tr>
<tr>
<td>Pancreatic cancer</td>
<td>460</td>
<td>1.71</td>
</tr>
<tr>
<td>Stomach cancer</td>
<td>322</td>
<td>1.16</td>
</tr>
<tr>
<td>Liver cancer</td>
<td>188</td>
<td>1.58</td>
</tr>
<tr>
<td>Lung cancer</td>
<td>2453</td>
<td>0.98</td>
</tr>
<tr>
<td>Kidney cancer</td>
<td>284</td>
<td>0.71</td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>139</td>
<td>0.93</td>
</tr>
<tr>
<td>Prostate cancer</td>
<td>1259</td>
<td>0.24</td>
</tr>
<tr>
<td>Breast cancer</td>
<td>202</td>
<td>0.53</td>
</tr>
<tr>
<td>All known primaries</td>
<td>6745</td>
<td>0.69</td>
</tr>
</tbody>
</table>

Riihimäki et al. BMC Cancer 2013, 13:36
CUP characteristics

- Regression/dormancy of the primary
  Autopsies fail to identify the primary in 30-40% of cases or identify small nodules in lung, pancreas. Why does it not grow?

- Early uncommon systemic metastases
  High volume mets in multiple sites. Viscera, skin, heart, scalp, kidneys, distant nodes

Pentheroudakis et al, CTR 2009;35:221-7
<table>
<thead>
<tr>
<th>Geographical area</th>
<th>Source</th>
<th>Frequency (%)</th>
<th>Period</th>
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<tbody>
<tr>
<td>USA</td>
<td>SEER</td>
<td>2.3</td>
<td>1973-1987</td>
</tr>
<tr>
<td>Australia</td>
<td>New South Wales Registry</td>
<td>4.2</td>
<td>1970-1990</td>
</tr>
<tr>
<td>Netherlands</td>
<td>Eindhoven Cancer Registry</td>
<td>4.0</td>
<td>1984-1992</td>
</tr>
<tr>
<td>Finland</td>
<td>IARC</td>
<td>2.5</td>
<td>-</td>
</tr>
<tr>
<td>Germany</td>
<td>-</td>
<td>7.8</td>
<td>1968-1984</td>
</tr>
<tr>
<td>Russia</td>
<td>-</td>
<td>3.6</td>
<td>-</td>
</tr>
<tr>
<td>Switzerland</td>
<td>Local registries</td>
<td>2.3</td>
<td>1984-1993</td>
</tr>
<tr>
<td>Japan</td>
<td>IARC</td>
<td>3.0</td>
<td>-</td>
</tr>
</tbody>
</table>
Incidence

1993-2011: European Age-Standardised Incidence Rates per 100,000

Average Number of New Cases Per Year and Age-Specific Incidence Rates per 100,000, UK 2009-2011

Prepared by Cancer Research UK - original data sources are available from http://www.cancerresearchuk.org/cancer-info/cancerstats
• Biopsy needed

• Generous material welcome (excisional biopsy or tru-cut rather than FNA)

• Classical histo work up with H&E stain
Primary markers

CK 7-/CK 20+
→ Colorectal and Merkel cell carcinoma

CK 7+/CK 20-
→ Lung, breast, thyroid, endometrial, cervical, and pancreatic carcinoma and cholangiocarcinoma

CK 7+/CK 20+
→ Urothelial, ovarian, and pancreatic cancer and cholangiocarcinoma

CK 7-/CK 20-
→ Hepatocellular, renal cell, prostate, squamous cell

Additional markers

CEA and CDX-2

TTF-1, ER, PR, GCDFP-15, and CK 19

Urothelin and WT-1

Hep Par-1 and PSA
Molecular Assays
<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 (%)</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 Tissue of Origin test</td>
<td>cDNA microarray</td>
<td>Frozen/FFPE</td>
<td>15</td>
<td>1500</td>
<td>89</td>
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<tr>
<td>Rosetta Genomics MiRview mets-2</td>
<td>Microarray miRNA</td>
<td>FFPE</td>
<td>42</td>
<td>64 miRNAs</td>
<td>92</td>
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<tr>
<td>bioTheranostics CancerType ID</td>
<td>RT-PCR mRNA</td>
<td>FFPE</td>
<td>39 (including subtypes)</td>
<td>92</td>
<td>86</td>
</tr>
</tbody>
</table>
IDENTIFICATION OF PRIMARY SITE BY GENETIC PROFILING (MICROARRAYS) FROM ALL PUBLISHED CUP SERIES

Years of Publications : 2005-2007

No of Samples : > 500 (cDNA)

Biological Assignment of Primaries (Accuracy) : 50 – 87 %

Primary Sites Identified :

- Breast : 15 %
- Pancreas : 12.5 %
- Bowel : 12 %
- Lung : 11.5 %
- Genital system : 9 %
- Liver/bile duct : 8 %
- Kidney / adrenals : 6 %
- Bladder / ureter : 5 %
- Stomach : 3 %
- Other : 18 %

Fig. 2 – Relative proportion of molecularly-assigned primaries in published series.

Eur J Cancer 2026-36, 2007
H&E morphologic evaluation shows a poorly differentiated or undifferentiated cancer; clinical presentation not helpful; limited biopsy with difficult access to more tissue

- Keep tissue (2–3 cellular slides) aside for potential ToO profiling
- Proceed with a few (6-7) pertinent IHCs based on morphology

- No result with IHC: proceed to ToO MP instead of additional IHC
  - No result with MP: consider next-generation sequencing
  - Additional directed IHC to seek concordance with MP
CLINICAL AND THERAPEUTIC UTILITY OF GENE AND PROTEIN MICROARRAY TECHNOLOGIES

QUESTION 1

DOES MOLECULAR ASSAYS, INCREASE THE ACCURACY OF IDENTIFYING THE PRIMARY SITE?

ANSWER 1

YES: UP TO 90% ACCURACY

QUESTION 2

DOES THIS DIAGNOSTIC AID RESULTS IN IMPROVEMENT OF PATIENT OUTCOME?

ANSWER 2

?
## CUP Staging

<table>
<thead>
<tr>
<th>Assessment suggested</th>
<th>Target patient population</th>
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<tbody>
<tr>
<td>Thorough medical history and physical examination</td>
<td>All patients</td>
</tr>
<tr>
<td>Basic blood and biochemistry survey</td>
<td>All patients</td>
</tr>
<tr>
<td>CT scans of thorax, abdomen and pelvis</td>
<td>All patients</td>
</tr>
<tr>
<td>Mammography</td>
<td>Female patients</td>
</tr>
<tr>
<td>Work-up for CUP subsets</td>
<td>Female with axillary adenocarcinoma</td>
</tr>
<tr>
<td>Breast MRI</td>
<td>Patients with midline metastatic disease</td>
</tr>
<tr>
<td>Serum α-fetoprotein and human chorionic gonadotropin</td>
<td>Male with adenocarcinomatous bony metastases</td>
</tr>
<tr>
<td>Serum prostate-specific antigen</td>
<td></td>
</tr>
<tr>
<td>Head and neck CT/PET scan (optional)</td>
<td>Cervical squamous carcinoma</td>
</tr>
<tr>
<td>Endoscopies</td>
<td>Sign/symptom/lab-oriented</td>
</tr>
<tr>
<td>Octreoscan and plasma chromogranin A</td>
<td>Patients with neuroendocrine tumor CUP</td>
</tr>
</tbody>
</table>
SERUM TUMOR MARKERS

- Routine evaluation of current commonly used markers have not been proven of any prognostic or diagnostic assistance.

- A non-specific multiple overexpression of the adenocarcinoma markers (CEA, CA 125, CA 15-3, CA 19-9) has been observed in the majority of CUP patients.

- **Worthwhile to request:**
  - **PSA** in men with bone metastatic adenocarcinoma
  - **B-HCG & AFP** in men with an undifferentiated tumor
  - **AFP** in patients with hepatic tumors
  - **CA 125** women with papillary adenocarcinoma of peritoneal cavity.
  - **CA 15-3** women with adenocarcinoma involving only axillary lymph nodes.
CUP

FAVOURABLE OR GOOD PROGNOSIS SUBSETS
20%

UNFAVOURABLE OR POOR PROGNOSIS SUBSETS
80%
1. Poorly differentiated carcinoma with **midline distribution** (extragonadal germ cell syndrome).

2. Women with **papillary** adenocarcinoma of peritoneal cavity.

3. Women with adenocarcinoma involving only **axillary** lymph nodes.

4. **Squamous** cell carcinoma involving cervical lymph nodes.

5. Poorly differentiated **neuroendocrine** carcinomas.

6. Men with **blastic bone** metastases PSA+ (adenocarcinoma).

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

8. Isolated **inguinal** adenopathy (squamous carcinoma).

CHARACTERISTICS OF PATIENTS WITH POORLY DIFFERENTIATED CUP

GENDER / AGE : Men / < 50 yrs

TUMOR INVOLVEMENT : Mediastinum
Retroperitoneum
Lungs
Lymph nodes

TUMOR MARKERS : Elevated serum levels of β-HGC or AFP

CLINICAL EVOLUTION : Rapid tumor growth

RESPONSE TO Rx : Favourable response to Cisplatin - based chemotherapy. RR 50% (CRs: 15-25%)

SURVIVAL : Median : 13 months
15% long – term survivors
PERITONEAL CARCINOMATOSIS IN FEMALES

THE NATURAL HISTORY

**Incidence**  
10% of CUP patients

**Mean Age (yrs)**  
60 (25 – 80)

**Clinical Picture**  
Abdominal distension, pelvic masses, ascites

**Surgical Picture**  
Abdominal masses, peritoneal disease, ascites, with normal ovaries

**Histology**  
Papillary serous carcinoma (± psammoma bodies)

**Serum CA-125**  
Often abnormal or markedly elevated.
WOMEN WITH PAPILLARY ADENOCARCINOMA OF PERITONEAL CAVILY
(Peritoneal Adenocarcinomatosis)

**Treatment:**
- As FIGO III ovarian cancer.
- Surgical cytoreduction.
- Platinum – based chemotherapy.

**Response Rate:**
40 – 60 % (CR : 30 %)

**Survival:**
Median : 16 months

**Long – term survival:**
5-yr: 10 %
<table>
<thead>
<tr>
<th><strong>Years</strong></th>
<th>: 1975 – 2006 (24 studies)</th>
</tr>
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<tbody>
<tr>
<td><strong>N</strong></td>
<td>: 689 patients</td>
</tr>
<tr>
<td><strong>Mean Age</strong></td>
<td>: 52 yr</td>
</tr>
<tr>
<td><strong>Menopause status</strong></td>
<td>: Postmenopausal 66%</td>
</tr>
<tr>
<td></td>
<td>Premenopausal 34%</td>
</tr>
<tr>
<td><strong>Histology</strong></td>
<td>: Ductal adenocarcinoma 83%</td>
</tr>
<tr>
<td></td>
<td>ER/PR 40 - 50/%, HER2 31%</td>
</tr>
<tr>
<td><strong>Nodal status</strong></td>
<td>: N1 : 48% &gt; N1 : 52</td>
</tr>
</tbody>
</table>
Treatment and Outcome

- Mastectomy / axillary dissection: 59%
- Primary breast irradiation: 26%
- Observation: 15%

- Logoregional recurrence rate: 25% (mostly in observation cases)

- 5-yr Survival: 72% (similar to stage II-III breast cancer)

- No survival difference between conservative management (breast preservation + RT) and mastectomy
SQUAMOUS CELL CANCER INVOLVING CERVICAL LYMPH NODES

**Treatment:**
- As locally advanced head-neck cancer.
- Surgery alone is inferior except pN1 neck disease with no extracapsular extension.
- **Radiation**: both sides of neck and mucosa (entire pharyngeal axis and larynx).
- **Chemotherapy** remains undefined (despite encouraging results with Platinum-based).

**Survival:**
- 5-year survival 35–50%.
- Documented long term disease–free survivors.
POORLY DIFFERENTIATED NEUROENDOCRINE CARCINOMAS

Treatment: Platinum – based or paclitaxel / carboplatin – based chemotherapy

Response: 50 – 70% (CR: 25%)

Survival: Median: 14.5 months
            3-yr: 24%
UNFAVOURABLE SUBSETS

1. Adenocarcinoma metastatic to the liver or other organs
2. Non-papillary malignant ascites (adenocarcinoma)
3. Multiple cerebral metastases (adeno or squamous Ca)
4. Multiple lung/pleural metastases (adenocarcinoma)
5. Multiple metastatic bone disease (adenocarcinoma)
6. Squamous – cell carcinoma of the abdominal cavity
<table>
<thead>
<tr>
<th>Author and Year of Publication</th>
<th>No. of Patients</th>
<th>Regimen</th>
<th>Median Survival (mo)</th>
<th>1-Year Survival (%)</th>
<th>2-Year Survival (%)</th>
<th>3-Year Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Briasoulis et al, 2000\textsuperscript{34}</td>
<td>33</td>
<td>PCb</td>
<td>10</td>
<td>25</td>
<td>5</td>
<td>NR</td>
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<tr>
<td>Dowell et al, 2001\textsuperscript{35}</td>
<td>34</td>
<td>P5FUL (17)</td>
<td>8.3</td>
<td>26</td>
<td>NR</td>
<td>NR</td>
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<tr>
<td></td>
<td></td>
<td>CbE (17)</td>
<td>6.4</td>
<td></td>
<td></td>
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<tr>
<td>Balaña et al, 2003\textsuperscript{38}</td>
<td>30</td>
<td>GCE</td>
<td>7.2</td>
<td>36</td>
<td>14</td>
<td>NR</td>
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<tr>
<td>Park et al, 2004\textsuperscript{40}</td>
<td>37</td>
<td>PC</td>
<td>11</td>
<td>38</td>
<td>11</td>
<td>NR</td>
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<tr>
<td>Piga et al, 2004\textsuperscript{39}</td>
<td>102</td>
<td>CbDoxE</td>
<td>9</td>
<td>35.3</td>
<td>18</td>
<td>11</td>
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<td>Pouessel et al, 2004\textsuperscript{41}</td>
<td>35</td>
<td>GD</td>
<td>10</td>
<td>43</td>
<td>7</td>
<td>NR</td>
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<tr>
<td>El-Rayes et al, 2005\textsuperscript{43}</td>
<td>22</td>
<td>PCb</td>
<td>6.5</td>
<td>27</td>
<td>NR</td>
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<td>Pittman et al, 2006\textsuperscript{36}</td>
<td>51</td>
<td>GcB</td>
<td>7.8</td>
<td>26</td>
<td>12</td>
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<td>Palmeri et al, 2006\textsuperscript{44}</td>
<td>66</td>
<td>GPC (33)</td>
<td>9.6</td>
<td>30</td>
<td>NR</td>
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<td></td>
<td></td>
<td>GVC (33)</td>
<td>13.6</td>
<td>52</td>
<td>NR</td>
<td>NR</td>
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<tr>
<td>Berry et al, 2007\textsuperscript{46}</td>
<td>42</td>
<td>PCb</td>
<td>8.5</td>
<td>33</td>
<td>17</td>
<td>NR</td>
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<tr>
<td>Briasoulis et al, 2007\textsuperscript{42}</td>
<td>47</td>
<td>OxIr</td>
<td>9.5</td>
<td>40</td>
<td>NR</td>
<td>NR</td>
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<tr>
<td>Schneider et al, 2007\textsuperscript{45}</td>
<td>33</td>
<td>GCaCb</td>
<td>7.6</td>
<td>35.6</td>
<td>14.2</td>
<td>NR</td>
</tr>
<tr>
<td>MPCRN (5 trials) 1997-2008\textsuperscript{1,21-24}</td>
<td>396</td>
<td>Multiple regimens (see text)</td>
<td>9.1</td>
<td>38</td>
<td>19</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>928</td>
<td></td>
<td></td>
<td>8.9*</td>
<td>34.6*</td>
<td>13*</td>
</tr>
</tbody>
</table>
OVERALL RESULTS OF CHEMOTHERAPY IN CUP PATIENTS WITH LIVER METASTASES

- Nº of patients: 711
- Response rate: < 20%
- Median survival: 5.5 months
A poor-risk CUP subgroup that may not be so

Varadhachary et al, Int J Clin Oncol 2013

MedOS
37 m
21 m
PROGNOSTICATION AND TREATMENT
1. First Parameter: CUP Clinicopathologic Subgroup

- Serous Peritoneal, Axillary Nodal, Squamous Head Neck.
  
  **SCORE 0**
  
  *Median OS 36 months*
  
  *No further scoring prognostication*

- Nodal, Neuroendocrine, Mucinous Peritoneal.
  
  **SCORE POINTS 1**

- Visceral.
  
  **SCORE POINTS 2**

2. Second Parameter: Leukocytosis (WBC > 10,000/mm3)

- **YES**
  
  **SCORE POINT 0**

- **NO**
  
  **SCORE POINT 0**

- **YES**
  
  **SCORE POINT 1**

3. Third Parameter: PS 0 or 1

**SUMMARY OF RISK GROUPS AND MEDIAN SURVIVAL**

- **SCORE 0**  **MEDIAN OS 36 months**  **LOW RISK**
- **SCORE 1**  **MEDIAN OS 14 months**  **INTERMEDIATE**
- **SCORE 2**  **MEDIAN OS 11 months**  **RISK**
- **SCORE 3**  **MEDIAN OS 8 months**  **HIGH**
- **SCORE 4**  **MEDIAN OS 5 months**  **RISK**
<table>
<thead>
<tr>
<th>Chemotherapy (mg/m²)</th>
<th>Time</th>
<th>Interval</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cisplatin 60–75</td>
<td>Day 1</td>
<td>Q 3 weeks</td>
<td>Fit patients, adequate hydration</td>
</tr>
<tr>
<td>Gemcitabine 1000</td>
<td>Day 1 + 8</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cisplatin 75</td>
<td>Day 1</td>
<td>Q 3 weeks</td>
<td>Fit patients with neuroendocrine feature-CUP, adequate hydration</td>
</tr>
<tr>
<td>Etoposide 100</td>
<td>Days 1–3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paclitaxel 175</td>
<td>Day 1</td>
<td>Q 3 weeks</td>
<td>Convenient outpatient regimen, monitor neurotoxicity</td>
</tr>
<tr>
<td>Carboplatin AUC 5</td>
<td>Day 1</td>
<td>Q 3 weeks</td>
<td></td>
</tr>
<tr>
<td>Docetaxel 75</td>
<td>Day 1</td>
<td>Q 3 weeks</td>
<td>Convenient outpatient regimen, monitor neurotoxicity</td>
</tr>
<tr>
<td>Carboplatin AUC 5</td>
<td>Day 1</td>
<td>Q 3 weeks</td>
<td></td>
</tr>
<tr>
<td>Irinotecan 160</td>
<td>Day 1</td>
<td>Q 3 weeks</td>
<td>Outpatient regimen, monitor for neurotoxicity and diarrhea</td>
</tr>
<tr>
<td>Oxaliplatin 80</td>
<td>Day 1</td>
<td>Q 3 weeks</td>
<td></td>
</tr>
<tr>
<td>Oral Capecitabine 2000 ± Oxaliplatin 85–130</td>
<td>Days 1–14</td>
<td>Q 3 weeks</td>
<td>Outpatient regimen, risk for diarrhea and neurotoxicity</td>
</tr>
<tr>
<td>Gemcitabine 1000/Irinotecan 100</td>
<td>Day 1 + 8</td>
<td>Q 3 weeks</td>
<td>Convenient outpatient regimen, monitor diarrhea</td>
</tr>
</tbody>
</table>
Multiple-treatments meta-analysis results for death.

<table>
<thead>
<tr>
<th></th>
<th>HR(^a)</th>
<th>95% CrI</th>
</tr>
</thead>
<tbody>
<tr>
<td>nPnTc vs. nPnTm</td>
<td>1.01</td>
<td>0.59–1.72</td>
</tr>
<tr>
<td>Platinum vs. nPnTm</td>
<td>0.69</td>
<td>0.39–1.28</td>
</tr>
<tr>
<td>Taxane vs. nPnTm</td>
<td>0.66</td>
<td>0.22–2.08</td>
</tr>
<tr>
<td>Platinum plus taxane vs. nPnTm</td>
<td>0.81</td>
<td>0.34–1.89</td>
</tr>
<tr>
<td>Platinum vs. nPnTc</td>
<td>0.69</td>
<td>0.43–1.15</td>
</tr>
<tr>
<td>Taxane vs. nPnTc</td>
<td>0.66</td>
<td>0.23–2.00</td>
</tr>
<tr>
<td>Platinum plus taxane vs. nPnTc</td>
<td>0.80</td>
<td>0.39–1.67</td>
</tr>
<tr>
<td>Taxane vs. platinum</td>
<td>0.95</td>
<td>0.37–2.5</td>
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<tr>
<td>Platinum plus taxane vs. platinum</td>
<td>1.16</td>
<td>0.56–2.38</td>
</tr>
<tr>
<td>Platinum plus taxane vs. taxane</td>
<td>1.22</td>
<td>0.36–4.00</td>
</tr>
</tbody>
</table>

\(^a\) A hazard ratio (HR) above one means that the risk of death is higher with the first rather than second listed regimen; nPnTm, non-platinum, non-taxane monotherapy; nPnTc, non-platinum, non-taxane combination.
• SHOULD WE TARGET CUP GENETIC ABERRATIONS?

SHOULD WE ADMINISTER TARGETED THERAPY ACCORDING TO THE PRIMARY SITE?
Actionable genetic alterations in CUP-1

- N=1350 patients with CUP
- Caris Life Sciences, Phoenix, USA
- Mutational analyses, in-situ hybridisation, immunohistochemistry, (RT)-qPCR.

- Actionable biomarkers were identified in 77%:
  - Steroid receptor IHC expression
  - MET IHC expression
  - PTEN loss of IHC expression,
  - Activating mutations in EGFR, BRAF, PIK3CA
  - Gene copy number variations in HER2, TOP2A and MET.

Abstract E17-7084), ECCO/ESMO European Cancer Congress 2013
Targeted next-generation sequencing (NGS) of carcinoma of unknown primary site (CUP): Actionable genomic alterations (GA) and new routes to targeted therapies. Jeffrey S. Ross, Kai Wang, Geoff Otto, Gary A. Palmer, et al
J Clin Oncol 32:5s, 2014 (suppl; abstr 11048)

- Hybridization capture of exons/introns from 236 cancer-related genes was applied to DNA extracted from 200 CUP FFPE specimens.
- 169 (85%) CUP had at least 1 actionable GA (2.00 actionable GA/CUP).
- The most common actionable GA were KRAS (25%), CDKN2A (19%), MCL1 (8-10%), PTEN (8%), PIK3CA (8%), BRAF (6% in ACUP and 2% in non-ACUP) and NF1 (5%).
- Mutations, amplifications and rearrangements of ERBB2 (10%), EGFR (8%) and BRAF (6%) were common in ACUP but not present in non-ACUP.
Molecular gene expression profiling to predict the tissue of origin and direct site-specific therapy in patients with carcinoma of unknown primary site (CUP): Results of a prospective Sarah Cannon Research Institute Trial

F. Anthony Greco, MD¹,²; Mark S. Rubin, MD¹,³; David R. Spigel, MD¹,²; Samuel Raby¹; Thabiso Chirwa¹; Raven Quinn, MS¹; Catherine A. Schnabel, Ph.D.⁴; Mark G. Erlander, Ph.D.⁴; John D. Hainsworth, MD¹,²

¹Sarah Cannon Research Institute (SCRI), Nashville, TN; ²Tennessee Oncology, PLLC, Nashville, TN; ³Florida Cancer Specialists/SCRI, Ft Myers, FL; ⁴bioTheranostics, Inc., San Diego, CA

bioTheranostics CancerTypeID 92-gene test

Presented at the 2012 ASCO Annual Meeting. Presented data is the property of the author.
Patients enrolled
N = 289

Successful assay
N = 252

Insufficient tissue for assay
N = 37
Off study

Candidate for treatment
N = 223

Not a treatment candidate
N = 29
Off study

Received empiric CUP therapy
N = 29

Received site-specific therapy directed by assay results
N = 194

Received site-specific therapy for more responsive tumor types
N = 115

Received site-specific therapy for less responsive tumor types
N = 79
# Survival in 223 Treated Patients and in Subsets

<table>
<thead>
<tr>
<th>Patient Group</th>
<th>Number</th>
<th>Median survival (mo.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All treated</td>
<td>223</td>
<td>10.8</td>
</tr>
<tr>
<td>Assay-directed treatment</td>
<td>194</td>
<td>12.5, p=0.02</td>
</tr>
<tr>
<td>Empiric treatment</td>
<td>29</td>
<td>4.7</td>
</tr>
<tr>
<td><strong>Tumor type</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Treatment responsive</td>
<td>115</td>
<td>13.4, p=0.04</td>
</tr>
<tr>
<td>Less treatment responsive</td>
<td>79</td>
<td>7.6</td>
</tr>
<tr>
<td><strong>Individual tumor types</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biliary tract</td>
<td>45</td>
<td>6.8</td>
</tr>
<tr>
<td>Pancreas</td>
<td>12</td>
<td>8.2</td>
</tr>
<tr>
<td>Colorectal</td>
<td>26</td>
<td>12.5</td>
</tr>
<tr>
<td>NSCLC</td>
<td>23</td>
<td>15.9</td>
</tr>
<tr>
<td>Ovary</td>
<td>10</td>
<td>29.6</td>
</tr>
<tr>
<td>Breast</td>
<td>10</td>
<td>NYR (&gt;24)</td>
</tr>
</tbody>
</table>

NYR = not yet reached; *Includes 194 patients who received assay-directed treatment
Real life questions

• Will Molecular Profiling Assays improve patient outcome by administration of Primary Tissue-specific therapy?

• Does a CUP biologically assigned as X Cancer behave similarly to Typical Metastatic X cancer?

• What about comparing CUP metastases to matched Known Primary Metastases (KPM)?
More

• Should we screen for actionable Genetic Aberrations in CUP?
• If we find such, how do we gain access to the targeted agents?
• Even if we have access to the targeted agents, how do we know they will work?
• How can we ignore the microenvironment and systems biology?
• Example: Targeting mutant BRAF in melanoma vs Colorectal Cancer
Angiogenesis in CUP

- Rashid et al, ASCO 2005:
  IHC VEGF expression in 49% of 76 CUP.

- Karavasilis et al, BMC Cancer 2005:
  N=81.
  IHC VEGF expression in 100%.

- Angiogenesis is active in CUP, though this is a feature common for metastatic solid tumours in general.
### Mutational profiling in CUP

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Category</th>
<th>HR</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N=87</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KRAS exon 2</td>
<td>Activating mutations: 9 (10.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inactivating mutations: 2 (2.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRAF exon 15</td>
<td>Activating mutations: 4 (4.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inactivating mutations: 1 (1.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PIK3CA exons 9, 20</td>
<td>Activating mutations: 6 (6.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inactivating mutations: 2 (2.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CTNNB1 exons 1,3,5</td>
<td>Activating mutations: 17 (19.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inactivating mutations: 1 (1.2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MET exon 18</td>
<td>Activating mutations: 4 (4.5%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inactivating mutations: 2 (2.2%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Parameter**

- **HR** indicates hazard ratio, which measures the relative risk of an event occurring.
- **p** value indicates the statistical significance of the results. A p value less than 0.05 is considered statistically significant.

**Reference:**


**Note:**

- **Activating MET or CTNNB1 mutation**
  - Activating MET or CTNNB1 mutation suggests the presence of at least one activating mutation in these genes.
  - The absence of activating mutations in these genes is indicated.

**Example Calculation:**

- For **MET_exon_18**, where an inactivating mutation is present:
  - The HR is 0.43, with a p value of 0.34.
  - For **CTNNB1_exons_1_3_5**, where an inactivating mutation is present:
  - The HR is 0.20, with a p value of 0.001.
Hypoxia

- Koo J et al, Cancer Cell Biol 2010
- TMA IHC study of hypoxia related proteins in 69 CUP
- Hypoxia phenotype (GLUT1, HIF1α, COX2) present in 25% of CUP, associated with poor prognosis
Metabolic phenotypes of CUP

• N=77 CUP

• TMA IHC expression of metabolic enzymes

  GLYCOLYSIS Glut-1, CA IX, MCT4;
  GLUTAMINOLYSIS GLS1, GDH, ASCT2;
  MITOCHONDRIAL ATP synthase, SDHA, SDHB

• More visceral metastases with glutaminolytic activity in tumour stroma (p=0.003)

• Kim HM, Journal of Translational Medicine 2014, 12:2
3. EPITHELIAL MESENCHYMAL TRANSITION AND STEMNESS in 100 CUP

• IHC:

  EMT phenotype:
  
  E-Cadherin, Snail, N-Cadherin, Vimentin
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>EMT present</th>
<th>EMT absent</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender</td>
<td>8 (17.4)</td>
<td>38 (82.6)</td>
<td>0.002</td>
</tr>
<tr>
<td>Female gender</td>
<td>0 (0)</td>
<td>52 (100)</td>
<td></td>
</tr>
<tr>
<td>High grade</td>
<td>6 (13.6)</td>
<td>38 (86.4)</td>
<td>0.05</td>
</tr>
<tr>
<td>Low grade</td>
<td>2 (4.5)</td>
<td>42 (95.5)</td>
<td></td>
</tr>
<tr>
<td>Visceral metastases</td>
<td>2 (13.3)</td>
<td>13 (86.7)</td>
<td>0.05</td>
</tr>
<tr>
<td>Non-visceral mets</td>
<td>3 (5.1)</td>
<td>55 (94.9)</td>
<td></td>
</tr>
</tbody>
</table>

**Response**

| CR or PR        | 3 (7.5)     | 37 (92.5)  | 0.893 |
| NO CR or PR     | 3 (8.3)     | 33 (91.7)  |       |

**PFS(months)**

| Median          | 5           | 8           | 0.112 |
| 95% CI          | 3.0-7.0     | 5.3-10.7    |       |

**Survival(months)**

| Median          | 8           | 13          | 0.023 |
| 95% CI          | 5.2-10.7    | 9.8-16.1    |       |
| 1-year (%)      | 0           | 75          |       |

**EMT H-SCORE**

**PFS**

| Median (months) | 5           | 8           | 0.541 |
| 95% CI          | 5.6-6.3     | 5.4-10.5    |       |

**Survival**

| Median          | 9           | 14          | 0.074 |
| 95% CI          | 7.2-10.7    | 10.6-17.3   |       |
| 1-year (%)      | 7.7         | 72.3        |       |
SHALL WE FIND ANYTHING WE DO NOT SEE IN METASTATIC TUMOURS?

TMA PROFILING

- Profiling 100 CUP cases for protein and phosphoprotein expression of 12 key membrane receptors, signal transducers, transcription factors, hypoxia, and cell cycle regulators.


Overall survival in patients with CUP with both high pAKT and pMAPK IHC expression

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hazard Ratio for risk of death</th>
<th>95% CI</th>
<th>2-sided p</th>
</tr>
</thead>
<tbody>
<tr>
<td>P21</td>
<td>0.34</td>
<td>0.16 – 0.73</td>
<td>0.005</td>
</tr>
<tr>
<td>cMET</td>
<td>0.48</td>
<td>0.26-0.91</td>
<td>0.025</td>
</tr>
<tr>
<td>pAKT Thr308</td>
<td>2.39</td>
<td>1.23 – 4.66</td>
<td>0.01</td>
</tr>
<tr>
<td>pS6RP Ser235/236</td>
<td>2.76</td>
<td>1.31 – 5.84</td>
<td>0.008</td>
</tr>
</tbody>
</table>
miRNA CUP SIGNATURE

- N=150 CUP
- Compare the expression of a library of 900 microRNAs between:
  - CUPs biologically classified
  - Metastases from equivalent known primary tumours (KPM)

IS THERE A miRNA CUP SIGNATURE?

Conclusions

- No trace of a «CUP biologic signature», distinct from KPM

- **Activated pathways in CUP:**
  - Angiogenesis with concurrent hypoxia
  - Stromal glutaminolytic activity
  - AKT/S6RP axis with deficient apoptosis
  - b-Catenin/Wnt axis
  - MET axis
  - EMT activity

**Target them!**
• Is it simply metastases from a missed primary? Find the primary, administer primary tumour-specific therapy.

• Or is it truly a disease with a unique molecular signature that defines its metastatic propensity?

• Target the genetic aberrations.
<table>
<thead>
<tr>
<th>Trial</th>
<th>Sponsor</th>
<th>Design</th>
<th>Arms</th>
</tr>
</thead>
<tbody>
<tr>
<td>CUP ONE</td>
<td>NCRI/CRUK</td>
<td>RCT Phase II</td>
<td>ECX vs ECX+Vandetanib</td>
</tr>
<tr>
<td>MIRVIEW</td>
<td>TEVA/ROSETTA</td>
<td>Parallel Cohort</td>
<td>ClinPath work up vs ClinPath work up+MirView</td>
</tr>
<tr>
<td>UNKPRI20</td>
<td>Sarah Cannon RC, USA</td>
<td>Single arm Phase II</td>
<td>Therapy based on molecular profiling</td>
</tr>
<tr>
<td>UNKPRI21</td>
<td>Sarah Cannon RC, USA</td>
<td>RCT Phase II</td>
<td>Carboplatin Paclitaxel vs CP+Belinostat</td>
</tr>
<tr>
<td>GEFCAPI04</td>
<td>GEFCAPI, France</td>
<td>RCT Phase III</td>
<td>CDDP+Gemcitabine vs Pathwork test-based therapy</td>
</tr>
<tr>
<td>PACET-CUP</td>
<td>Ruprecht-Karls-University of Heidelberg</td>
<td>RCT Phase II</td>
<td>Carboplatin Paclitaxel vs CP+Cetuximab</td>
</tr>
<tr>
<td>CUP02</td>
<td>CRUK</td>
<td>RCT Phase III</td>
<td>ECX vs Molecularly Assigned Therapy</td>
</tr>
</tbody>
</table>
Evaluation and Management of Possible CUP Patient

Clinical Presentation

Initial Clinical/Diagnostic Evaluation and Biopsy

Standard Pathology, Immunohistochemistry Stains

Anatomical Primary Site Not Identified

Anatomical Primary Site Identified

Favorable Subset CUP

Specific Treatment for Subset

Molecular profile Assay on Selected Tumors (When IHC not diagnostic of a single tissue of origin)

Poor Risk: Additional Directed Evaluation

Single Tissue of Origin Diagnosed

Clinical Trial or Site Specific Therapy

Single Tissue of Origin Not Diagnosed

Clinical Trial or Empiric Therapy