Cancer of Unknown Primary (CUP)

• Diagnostic work-up: from immuno-histochemistry to molecular profiling

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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, which are mentioned here; their contribution to the study is intellectual and to provide molecular analyses but not otherwise financial.
• Dr Jayne Dennis (co-author of paper) declares no conflicts of interest.
Outline

• Cancer classification and CUP
• Diagnostic work-up of cancer in pathology
• Tissue-specific biomarker genes
• Immunohistochemistry for CUP
• Molecular profiling for CUP
  – Development, validation and application of tests
• Integration and conclusions
• (References: listed in paper)
Cancer classification

• Traditionally and now, cancer classification based on:
  – Cancer anatomical (primary) location, and
  – Cancer morphology
• These have long guided cancer patient management
• Establishing site, type and subtype, i.e. the diagnosis, is therefore a main aim of cancer pathology
• Tumour typing often possible on morphology alone
• CUP then requires classification by tumour typing in absence of known site: a systematic approach helps
Classification of cancer including CUP: A stepwise pathological approach

**Step 1: identify broad cancer type**
- Carcinoma
- Melanoma
- Lymphoma/leukaemia
- Sarcoma
- (Neuro-glial tumours)

**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)

**Step 3: if adenocarcinoma, predict primary site(s)**
- Lung
- Pancreas
- Colon
- Stomach
- Breast
- Ovary
- Prostate, etc
CUP: clinical and pathological problem

• Up to 15% of cancers present with metastases
  – In two-thirds or more, primary becomes evident
  – Up to one-third (5%) may become CUP

• CUP occurs mainly in solid organs, lymph nodes or serous cavities, so specimens are often small: core biopsies or cytology

• The term “CUP” equates to carcinoma: other main tumour types excluded by definition, but need considered in pathological work-up

www.esmo2012.org
CUP: common subtypes and sites

- In CUP, the common carcinoma subtypes are:
  - Adenocarcinoma: 60%
  - Squamous: 5%
  - Neuroendocrine: 5%
  - Poorly differentiated: 30%

- In CUP, the most common primary sites for adenocarcinoma are pancreas and lung; then colon, stomach and oesophagus, breast, ovary and prostate
CUP: classification for clinical benefit

• CUPs can be divided into good and poor prognosis groups; and specific treatment is available for certain CUP groups

• Aim of pathology in CUP is optimal tumour classification to enable identification of patients with good prognosis and/or treatable tumours

• Tumour classification should be flexible to incorporate advances: clinico-pathological discussion is vital

• How can we classify if morphology not diagnostic?
Example of difficult tumour
Tissue-specific or restricted genes

- 12,000 genes active in each tissue: >8,000 are widely expressed
- Minority of genes is tissue-specific or restricted, related to function i.e. differentiation
  - Regulatory genes e.g. TTF-1, CDX2, PAX8
  - Protein products e.g. PSA, CK7
  - Many markers shared by IHC and molecular profiling
Tissue-specific genes as cancer biomarkers

• Tumours are derived from specific tissues: normal tissue-specific morphology and gene expression is partly retained in cancer

• Shown by unsupervised clustering of profiles, when primary tumours, and most metastases, cluster by type/subtype and with corresponding normal tissue

• Residual gene expression is reduced in poorly differentiated tumours

• How are tissue-specific markers used in practice?
IHC performance and practice

• Some pathologists may diagnose confidently on morphology alone, others use IHC widely
• IHC is subjective: technical performance and microscopic interpretation varies, hence IHC biomarkers used in panels
• IHC is selective: tissue and time limited so a few biomarkers can be tested (7-8 is usual in CUP)
• In CUP, one barrier to correct tumour classification is simply not using the most appropriate markers: so careful biomarker selection is important

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

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carcinoma</td>
<td>Cytokeratins and other epithelial markers e.g. <strong>AE1/3</strong>, CK7, CK20, CK5, EMA</td>
</tr>
<tr>
<td>Melanoma</td>
<td><strong>S100</strong>, Melan-A, HMB45</td>
</tr>
<tr>
<td>Lymphoma/leukaemia</td>
<td><strong>CLA</strong>, CD20, CD3, CD138, CD30 etc.</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>Vimentin, actin, desmin, S100, c-kit etc</td>
</tr>
</tbody>
</table>
Immunohistochemistry for CUP: Step 2: if carcinoma, identify subtype

<table>
<thead>
<tr>
<th>Tumour Type</th>
<th>Markers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adenocarcinoma</td>
<td>CK7, CK20, PSA and other adenocarcinoma markers</td>
</tr>
<tr>
<td>Squamous carcinoma</td>
<td>CK5, p63</td>
</tr>
<tr>
<td>Transitional carcinoma</td>
<td>CK7, CK20, urothelin</td>
</tr>
<tr>
<td>Neuroendocrine carcinoma</td>
<td>Chromogranin, CD56, synaptophysin, TTF1</td>
</tr>
<tr>
<td>Solid carcinoma, renal</td>
<td>RCC, CD10, PAX8, Napsin A</td>
</tr>
<tr>
<td>Solid carcinoma, liver</td>
<td>Hepar1, CD10, glypican-3</td>
</tr>
<tr>
<td>Solid carcinoma, thyroid</td>
<td>TTF1, thyroglobulin, PAX8</td>
</tr>
<tr>
<td>Solid carcinoma, adrenal</td>
<td>Melan-A, inhibin</td>
</tr>
<tr>
<td>(Germ cell tumour)</td>
<td>OCT4, PLAP, HCG, AFP</td>
</tr>
<tr>
<td>(Mesothelioma)</td>
<td>Calretinin, mesothelin, WT1, D2-40</td>
</tr>
</tbody>
</table>
IHC: Step 3: If adenocarcinoma, predict possible primary site(s)

<table>
<thead>
<tr>
<th></th>
<th>PSA or NNX3.1</th>
<th>TTF1 or Napsin A</th>
<th>GCDFP-15 or mammoglobin</th>
<th>WT1</th>
<th>PAX8</th>
<th>ER</th>
<th>CA125</th>
<th>Meso-thelin</th>
<th>CK7</th>
<th>CDX2 and/or CK20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prostate</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Lung</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-/+</td>
<td>-/+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Breast</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>+/-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ovary serous</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Ovary mucinous</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-/+</td>
<td>-/+</td>
<td>-</td>
<td>-/+</td>
<td>-/+</td>
<td>-/+</td>
<td>-</td>
</tr>
<tr>
<td>Pancreas</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+/-</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
<td>-</td>
<td>+/-</td>
</tr>
<tr>
<td>Stomach</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-/+</td>
<td>+/-</td>
<td>-</td>
<td>-/+</td>
</tr>
<tr>
<td>Colon</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-/+</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
</tbody>
</table>

+ = 90% or more, +/- = 50-90%, -/+ = 10-50%, - = 10% or less
Tumour is carcinoma with positive CA125
CUP: diagnostic difficulties

• After morphology, with IHC if needed, common diagnostic difficulties in tumour typing are with:
  – Limited tissue: small or necrotic samples
  – Poorly differentiated tumours
  – Adenocarcinoma without obvious primary site
    • Pancreatic, colorectal and gastro-oesophageal (and ovarian) often in pairs difficult to distinguish; and lung

• This is the unmet clinical need for molecular profiling to address
Performance of IHC for comparison with molecular profiling

• How does IHC perform in cancer classification?
• Only 5-6 studies identified in recent meta-analysis
• Sensitivity of IHC panels was consistent, around 82% in mixed primary and metastatic tumours and 66% in metastases alone
• Confirms metastases are harder to classify than primary tumours by IHC and sets baseline for comparison

Anderson et al 2010
Molecular profiling for CUP

• Large-scale profiling for CUP achieved at mRNA, miRNA, DNA and epigenetic levels.

• Three tests commercially available:
  – Pathwork Tissue of Origin (TOO)
  – bioTheranostics’ Cancer Type ID (CTID)
  – miRview mets2

• “Different tissue types have distinct RNA profiles”
Molecular profiling for CUP: development of tests

- Gene expression profiles generated for hundreds of different tumours
- Subset of discriminatory genes identified and used to build diagnostic algorithms
- Cancer classification test
- Validation in known tumours: metastatic and poorly differentiated are more realistic
- Comparison with IHC
- Application in CUP

Feedback leading to “second generation” tests
## Characteristics of molecular CUP tests

<table>
<thead>
<tr>
<th></th>
<th>bioTheranostics CancerType ID</th>
<th>miRview mets2</th>
<th>Pathwork Tissue of Origin test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Molecular basis</td>
<td>mRNA</td>
<td>miRNA</td>
<td>mRNA</td>
</tr>
<tr>
<td>Technology used</td>
<td>RT-PCR</td>
<td>microarray</td>
<td>microarray</td>
</tr>
<tr>
<td>No. of genes analysed</td>
<td>92 mRNAs</td>
<td>64 miRNAs</td>
<td>2000 mRNAs</td>
</tr>
<tr>
<td>No. of tumours in training set</td>
<td>2206</td>
<td>1282</td>
<td>2140</td>
</tr>
<tr>
<td>No. of tumour types/subtypes in training set</td>
<td>54 types/subtypes</td>
<td>42 types/subtypes</td>
<td>15 (17) classes based on 58 (69) types/subtypes</td>
</tr>
<tr>
<td>How is test reported?</td>
<td>One (or more) type/subtype with probability &gt;5%; &lt;5% probability excludes.</td>
<td>Two diagnoses, the same or different: from one of 42 types/subtypes or 7 combined classes</td>
<td>Similarity score (SS) for each of 15 classes, totalling 100. SS&gt;70 is likely; SS &lt; 5 excludes.</td>
</tr>
</tbody>
</table>

# Molecular profiling for CUP: validation in known tumours

<table>
<thead>
<tr>
<th>Test</th>
<th>Sensitivity</th>
<th>Tumours</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>bioTheranostics</strong></td>
<td><strong>87-82%</strong></td>
<td>Known primaries and metastases</td>
</tr>
<tr>
<td><strong>CancerType ID</strong></td>
<td><strong>83%</strong></td>
<td>Known primaries</td>
</tr>
<tr>
<td></td>
<td><strong>78-72%</strong></td>
<td>High-grade known metastases</td>
</tr>
<tr>
<td><strong>miRview mets2</strong></td>
<td>85%</td>
<td>Known primaries and metastases</td>
</tr>
<tr>
<td><strong>Pathwork</strong></td>
<td>95%</td>
<td>Difficult primaries and metastases</td>
</tr>
<tr>
<td><strong>Tissue of Origin test</strong></td>
<td>91%</td>
<td>Known metastases</td>
</tr>
<tr>
<td></td>
<td>94%</td>
<td>Effusion cytology</td>
</tr>
<tr>
<td></td>
<td><strong>87%</strong></td>
<td>Poorly or un-differentiated primaries</td>
</tr>
</tbody>
</table>

Molecular profiling for CUP: application in CUP

<table>
<thead>
<tr>
<th>CancerType ID</th>
<th>Pre-diction yielded?</th>
<th>Clinical agree-ment?</th>
<th>Tumours and comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indeterminate or unknown primary</td>
<td>91%</td>
<td></td>
<td>Indeterminate or unknown primary</td>
</tr>
<tr>
<td>Mainly poorly differentiated or metastatic; more rare tumours than expected e.g. cholangioca</td>
<td>78-74%</td>
<td></td>
<td>Mainly poorly differentiated or metastatic; more rare tumours than expected e.g. cholangioca</td>
</tr>
<tr>
<td>CUP: incorrect included lung</td>
<td>85-75%</td>
<td></td>
<td>CUP: incorrect included lung</td>
</tr>
<tr>
<td>Various CUP</td>
<td>92%</td>
<td></td>
<td>Various CUP</td>
</tr>
<tr>
<td>Brain CUP</td>
<td>88%</td>
<td></td>
<td>Brain CUP</td>
</tr>
<tr>
<td>CUP: most clinically appropriate, more sarcomas than expected</td>
<td>96%</td>
<td></td>
<td>CUP: most clinically appropriate, more sarcomas than expected</td>
</tr>
</tbody>
</table>

Molecular profiling for CUP: limitations

- 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
- TOO test has very good validation results, though:
  - Its 15 reporting classes lack some CUP differential diagnoses e.g. neuroendocrine
  - In CUP, sarcoma predicted more than expected

Pillai et al 2011, Beck et al 2011
Molecular profiling for CUP: strengths in comparison with IHC

- CTID was more sensitive than IHC in high-grade metastases for tumour typing (78% versus 68%)
- miRview mets2 agreed with the final diagnosis in 92% of CUPs compare with 70% for IHC
- These IHC sensitivities are similar to the meta-analysis and thus appear appropriate
- Molecular tests may be 10-20% more sensitive than optimal IHC, confirming their potential clinical utility

Molecular profiling for CUP: clinical impact: 1

• 107 well worked-up patients had poorly differentiated or metastatic cancer. TOO testing affected the working diagnosis as follows:
  – 14 still had site unspecified
  – 36 still had same specified site
  – 30 changed from unspecified to specified site
  – 24 changed specified site
  – 3 changed from specified to unspecified site

• Molecular profiling changed diagnosis in around 50% and changed patient management in 65%
Molecular profiling for CUP: clinical impact: 2

• Molecular profiling has an impact on diagnosis
• The impact of molecular profiling on patient outcome is less clear
• Patients with CUP tumours with colorectal profiles, and treated as such, have survival times resembling metastatic colorectal cancer more than CUP as a whole
• Further studies underway

Hainsworth et al 2012, Greco et al 2012
Molecular profiling for CUP: non- or pre-commercial tests

- Many described: most as single paper with initial validation, often including CUP
- Most show high sensitivity, 78-90%, like commercial tests.
- Some tests are based on mRNA and miRNA and aim to classify all likely tumours
- Others are more specific assays for tumour subsets
- Some assays are broad but based on methylation or high-throughput sequencing
- Some are more formally integrated with pathology work-up

Tothill et al 2005, Centeno et al 2010 and others
Further integration of tests

- IHC and molecular profiling use similar tissue-specific genes: IHC is more selective and subjective
- Tumours difficult to diagnose using morphology and IHC are often also difficult for molecular profiling
- But in already well worked-up poorly differentiated tumours, molecular profiling may:
  - Out-perform IHC by 10-20%
  - Change diagnosis in around 50%
  - Affect patient management in most
Conclusions: pathology and IHC

• Pathology, with IHC if needed, remains “gold standard” in tumour classification, especially where:
  – Tumour is primary and/or
  – Tumour is at least moderately differentiated and/or
  – Any IHC results are classical and/or
  – The clinical context is appropriate

• IHC for CUP is ever-improving; and guidelines (e.g. ESMO) are available on IHC panels and application
Conclusions: molecular profiling 1

• Molecular profiling could contribute to diagnosis of poorly differentiated and/or metastatic tumours, especially where:
  – Morphology and IHC equivocal or conflicting with clinical context
  – Diagnosis usually either truly unknown or includes multiple possible differentials
  – Limited tissue or time?
Conclusions: molecular profiling 2

• Molecular profiling results should be evaluated in context of pathology and clinical findings
• Wider use of molecular profiling may need high-level strategic healthcare discussion, for funding etc.
• Molecular profiling not currently recommended in NICE or ESMO guidelines
Future including research

• More studies needed on molecular tests:
  – Comparison with each other in known tumours
  – Singly and in comparison in CUP
  – Comparison with IHC
  – Clinical impact on diagnosis, management and outcome

• Molecular tests for prognosis and prediction of therapy emerging: role in CUP?

• Cancer classifications flexible: talk with pathologists
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