Likely site of origin versus actionable mutations: which is the best approach for treatment?

Update on Peter MacCallum Cancer Centre CUP studies and putative projects

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AUSTRALIA

Acknowledgements – People support  
Schofield: NHMRC CDA Fellowship  
Bowtell: NHMRC PSR Fellowship
Current approaches to treatment

- Only 8 phase II trials and 4 prospective observational series including 2 randomised trials

- Striking lack of evidence to guide treatment, except for subsets with suspected primary site (e.g., squamous cell carcinoma)

- Novel therapeutic approaches are urgently needed (CUPONE)

- A better understanding of the disease will underpin these approaches
Classification of carcinoma of unknown primary – Is this a distinct cancer?

1. Can we more effectively classify them?
   - Improved classification may lead to:
     - Shorter time to likely diagnosis – reduced cost and patient morbidity
     - Allow more appropriate treatment

2. Is their biology distinct from their ‘conventional’ counterparts?
   - Identifying shared biological characteristics may lead to:
     - Use of molecularly targeted treatments
1. Can we more effectively classify them?

- Improved classification may lead to:
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2. Is their biology distinct from their ‘conventional’ counterparts?

- Identifying shared biological characteristics may lead to:
  - Use of molecularly targeted treatments
Comprehensive clinical diagnostic work up

Full physical exam (breast, rectal and pelvic examination)
basic blood profile (FBE, CUE, LFT, LDH),
Gastroscopy and colonoscopy/FOBT (if possible gastrointestinal primary)
Chest x-ray and CT (thorax, abdomen and pelvis),
Mammography (women), PSA (men) +/- PET (SCC H&N),
Immunohistochemistry and circulating biomarkers
Other tests for specific symptoms/laboratory abnormalities

~20-30% success rate (we think)

Diagnostic workup of CUP patients can be lengthy, delays initiation of treatment and patients may deteriorate.
Array based tests

Pathwork Diagnostic test

CancerTypeID BioTheranostics (CUP-Print)

CUP Diagnostic Assay
➤ developed by Bowtell & Tothill with National Information and Communication Technology Australia and HealthScope Pathology.
Gene expression-based classifier

- BUILD AN EXPRESSION DATABASE OF KNOWN TUMOURS
- CREATE A CLASSIFIER THAT PREDICTS KNOWNNS
- TEST ON CUP OR OTHER DIFFICULT CASES
Developing a data set of expression profiles

• 250 primary and metastatic tumours (fresh frozen)

• 16 histological types

• 32 histological and molecular subtypes

Heat map of gene expression

- 399 metastatic cancers of known origin of 15 classes (FFPE)
- Whole-Genome DASL HT Assay
  <http://www.illumina.com/technology/whole_genome_dasl_assay.ilmn>
<table>
<thead>
<tr>
<th>Tumour Class</th>
<th>N</th>
<th>Correct call with first prediction</th>
<th>Correct call within first two predictions</th>
<th>Correct call within first three predictions</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Average</td>
<td></td>
<td>83%</td>
<td>89%</td>
<td>93%</td>
<td>98.5%</td>
</tr>
<tr>
<td>Bladder</td>
<td>13</td>
<td>85%</td>
<td>92%</td>
<td>92%</td>
<td>99.7%</td>
</tr>
<tr>
<td>Breast</td>
<td>56</td>
<td>79%</td>
<td>93%</td>
<td>96%</td>
<td>96.7%</td>
</tr>
<tr>
<td>Colorectal</td>
<td>48</td>
<td>85%</td>
<td>92%</td>
<td>96%</td>
<td>96.4%</td>
</tr>
<tr>
<td>Gastric</td>
<td>26</td>
<td>81%</td>
<td>85%</td>
<td>89%</td>
<td>99.2%</td>
</tr>
<tr>
<td>Kidney</td>
<td>14</td>
<td>93%</td>
<td>100%</td>
<td>100%</td>
<td>99.7%</td>
</tr>
<tr>
<td>Lung</td>
<td>30</td>
<td>83%</td>
<td>97%</td>
<td>97%</td>
<td>97.6%</td>
</tr>
<tr>
<td>Melanoma</td>
<td>50</td>
<td>78%</td>
<td>84%</td>
<td>88%</td>
<td>98.8%</td>
</tr>
<tr>
<td>Neuroendocrine</td>
<td>18</td>
<td>83%</td>
<td>89%</td>
<td>89%</td>
<td>98.7%</td>
</tr>
<tr>
<td>Ovarian</td>
<td>22</td>
<td>77%</td>
<td>96%</td>
<td>100%</td>
<td>98.4%</td>
</tr>
<tr>
<td>Pancreas</td>
<td>18</td>
<td>72%</td>
<td>78%</td>
<td>83%</td>
<td>97.7%</td>
</tr>
<tr>
<td>Prostate</td>
<td>19</td>
<td>89%</td>
<td>95%</td>
<td>95%</td>
<td>99.7%</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>29</td>
<td>76%</td>
<td>83%</td>
<td>86%</td>
<td>96.6%</td>
</tr>
<tr>
<td>SCC</td>
<td>27</td>
<td>93%</td>
<td>93%</td>
<td>93%</td>
<td>99.2%</td>
</tr>
<tr>
<td>Testi</td>
<td>8</td>
<td>88%</td>
<td>88%</td>
<td>88%</td>
<td>99.7%</td>
</tr>
<tr>
<td>Thyroid</td>
<td>21</td>
<td>91%</td>
<td>91%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>
### Application of classifier to CUP cases

#### Table 3. Summaries of clinical history and array predictions for unknown primary samples

<table>
<thead>
<tr>
<th>Disease presentation and histology</th>
<th>Differential at initial presentation</th>
<th>Array prediction and outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>P01328: 52-y-old female, no previous history. Extensive abdominal tumor. Adenocarcinoma.</td>
<td></td>
<td>Breast (100). Left supraclavicular fossa and axillary nodes developed within 2 mo of chemotherapy.</td>
</tr>
<tr>
<td>P01698: 37-y-old female, no previous history. Pelvic mass, ascites, and left pleural effusion. Moderately differentiated adenocarcinoma with occasional signet ring features.</td>
<td>Pathologist thought that morphology strongly suggested nonovarian origin (e.g., gastric, colorectal, pancreas, or lung). Clinical picture consistent with ovarian cancer.</td>
<td>Ovarian (92). Treated with taxol/carboplatin for presumed ovarian primary. Good clinical response with normalization of CA125</td>
</tr>
<tr>
<td>P01946: 49-y-old female smoker, no previous history. Liver, bone, adrenal, and mediastinal disease. Atypical infiltrating epithelial cells forming glandlike structures.</td>
<td>Lung, colorectal.</td>
<td>Lung (60)</td>
</tr>
</tbody>
</table>

High confidence 11/13 cases
Current state of play

- Validation of the classifier with a large series of CUP (n>100) and known (n>200) metastatic tumours.

- Planned release date for assay is mid 2012.
Actionable Mutations

1. Can we more effectively classify them?
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2. Is their biology distinct from their ‘conventional’ counterparts?
   - Identifying shared biological characteristics may lead to:
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Proof of concept in known solid tumours

Tisimberido (2011) M. D. Anderson Cancer Center

- A personalized medicine program with 955 consecutive patients with advanced cancer
- Tumour molecular analysis was feasible in 852 (89%)
- 354 (41.5%) had ≥ 1 aberration detected.
- Matching patients with targeted drugs resulted in:
  - longer time to treatment failure (TTF) compared to their prior therapy
  - higher rates of response, survival and TTF compared to standard care.
Examples of Potential Targets for CUP

Tyrosine kinase MET
• controls cellular motility
• mutated in about 30% of poorly differentiated CUP samples vs 4% of unselected cancer samples (Stella et al, 2010)

RET and ALK
• In 40 colorectal and 24 non-small cell lung cancers genomic alterations in were identified in the target genes (RET and ALK) of 59% of patients.
  ➢ Crizotinib is a small molecule inhibitor of ALK and MET

Epidermal Growth Factor Receptor (EGFR)
• Over expression of EGFR has been reported in 66% of CUP cases, although few activating point mutations in EGFR-family members have been found to date

Aims

(i) To screen the 40-50 genes for which targeted agents currently exist to determine the frequency of actionable mutations

(ii) To explore another ~750 genes that are potential drivers of CUP biology.
Selection of targeted genes

- Targeted Hybridisation Capture
- SureSelect Human Kinome (Agilent)
- Additional 190 genes selected from the Cancer Genome Census (Sanger)
- Hand selected genes includes baits tiled across intronic regions frequently site of fusion breakpoints for 13 common oncogenic fusions

 ➤ Total genes = 817
Methods

• Targeted exome pulldown using Aligent solution-phase capture reagents followed by next generation DNA sequencing on an Illumina HiSeq 2000

• Somatic mutations will be detected by comparing germline (blood) and tumour DNA sequences

• Pathogenic germline mutations may also be identified
Future directions

- Three sources of information to direct treatment approaches:
  - Clinical diagnostic assessment ( Likely site of origin)
  - Gene expression-based classifier ( Likely site of origin)
  - Genetic analysis (Actionable mutations)
- May imply convergent or divergent approaches to treatment
- How can we combine information relating to likely site of origin with actionable mutations for best treatment approach?
- How to design an ethically responsible clinical trial with this information?
SUPER (Solving Unknown Primary cancer)

- Cohort recruitment
- Clinical protocol development
- Psychosocial studies
- Actionable mutations

CUP diagnostic test
Tothill et al (Can Res 2005)
Cohort ascertainment

CUP PATIENT ASCERTAINMENT

Peter Mac CUP clinic → Cancer2015 → SUPER cohort ← CUPONE – UK*

Clinical collaborators (RPAH, FMC) ← Healthscope (CUP diagnostic test)

* Biospecimens only
Conclusions

• CUP presents unique clinical opportunity to develop molecularly-guided approaches as first line therapy.

• If frequent actionable mutations are found, then the trick will be **how** to design a trial to test the value of targeted therapies.
Our Team

Chief Investigators
Schofield  Behavioural science
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Mileshkin  Medical Oncology
Waring  Pathology
deFazio  Cancer Cell Biology
Tattersall  Medical Oncology
Karapetis  Medical Oncology
Richardson  Medical Oncology

Associate Investigators
Barrett  Consumer Advocate
Bryant  Consumer Advocate & Nurse
Gooden  Nurse
Thomas  Medical Oncology
Mitchell  Medical genetics
Wasan  Medical Oncology
Lipton  Medical oncology
Ashley  Medical Oncology
Tothill  Molecular Biology
Zalcberg  Medical Oncologist
Lorgelly  Health Economics & quality of life