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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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 (eg 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

Likely Site of Origin

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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

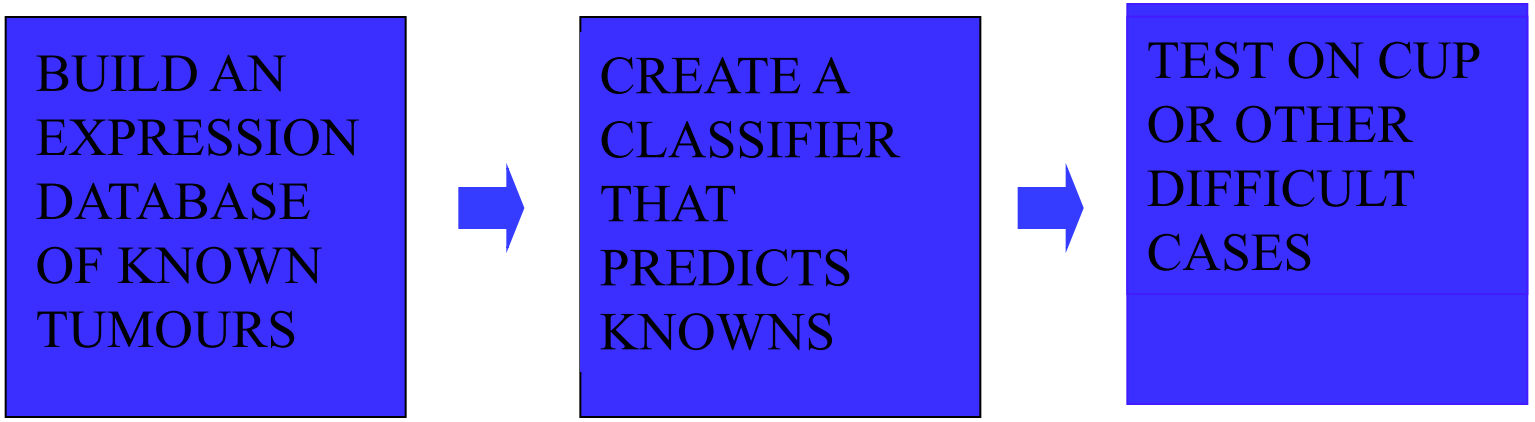
Pathwork Diagnostic test

CancerTypeID BioTheranostics (CUP-Print)

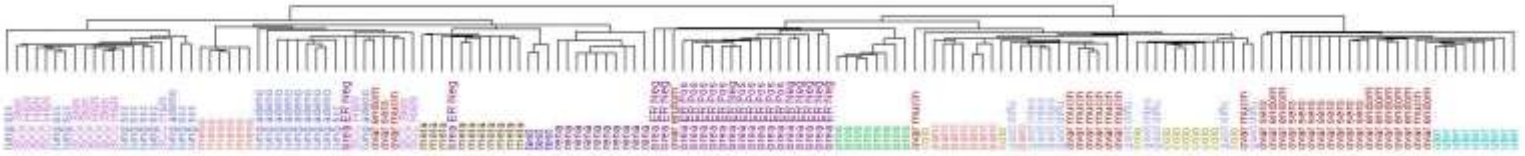
CUP Diagnostic Assay

- developed by Bowtell & Tothill with National Information and Communication Technology Australia and HealthScope Pathology.

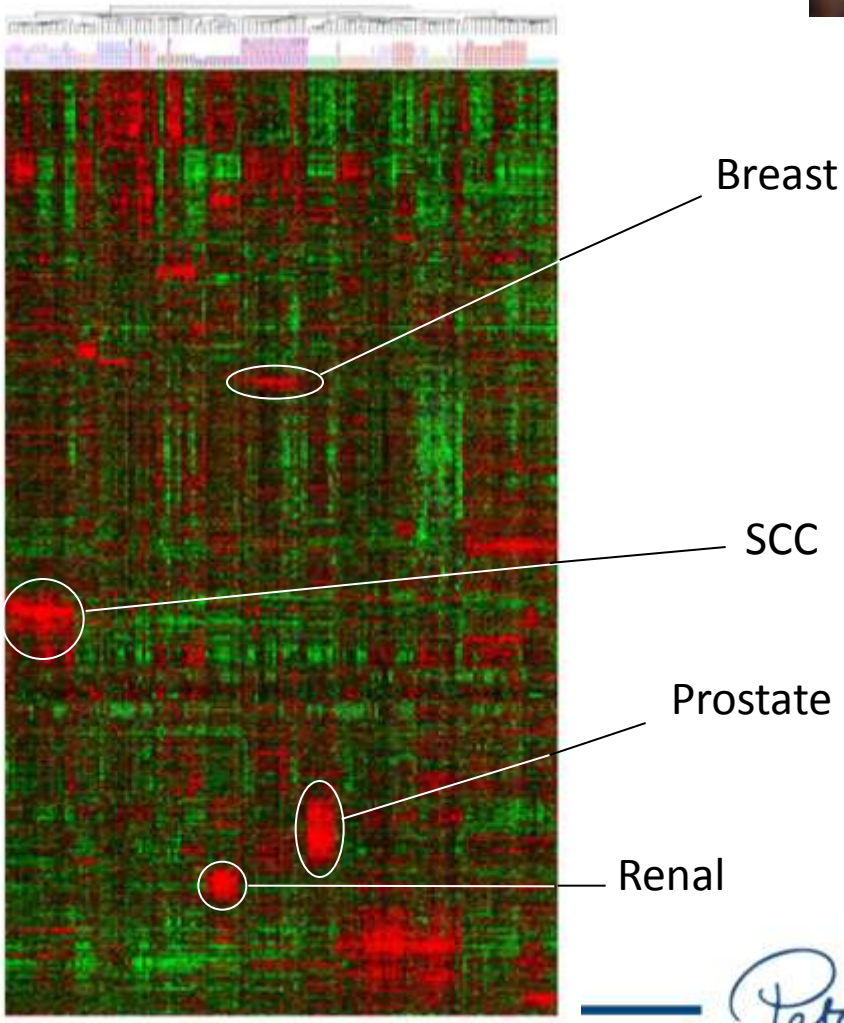
Gene expression-based classifier



Developing a data set of expression profiles



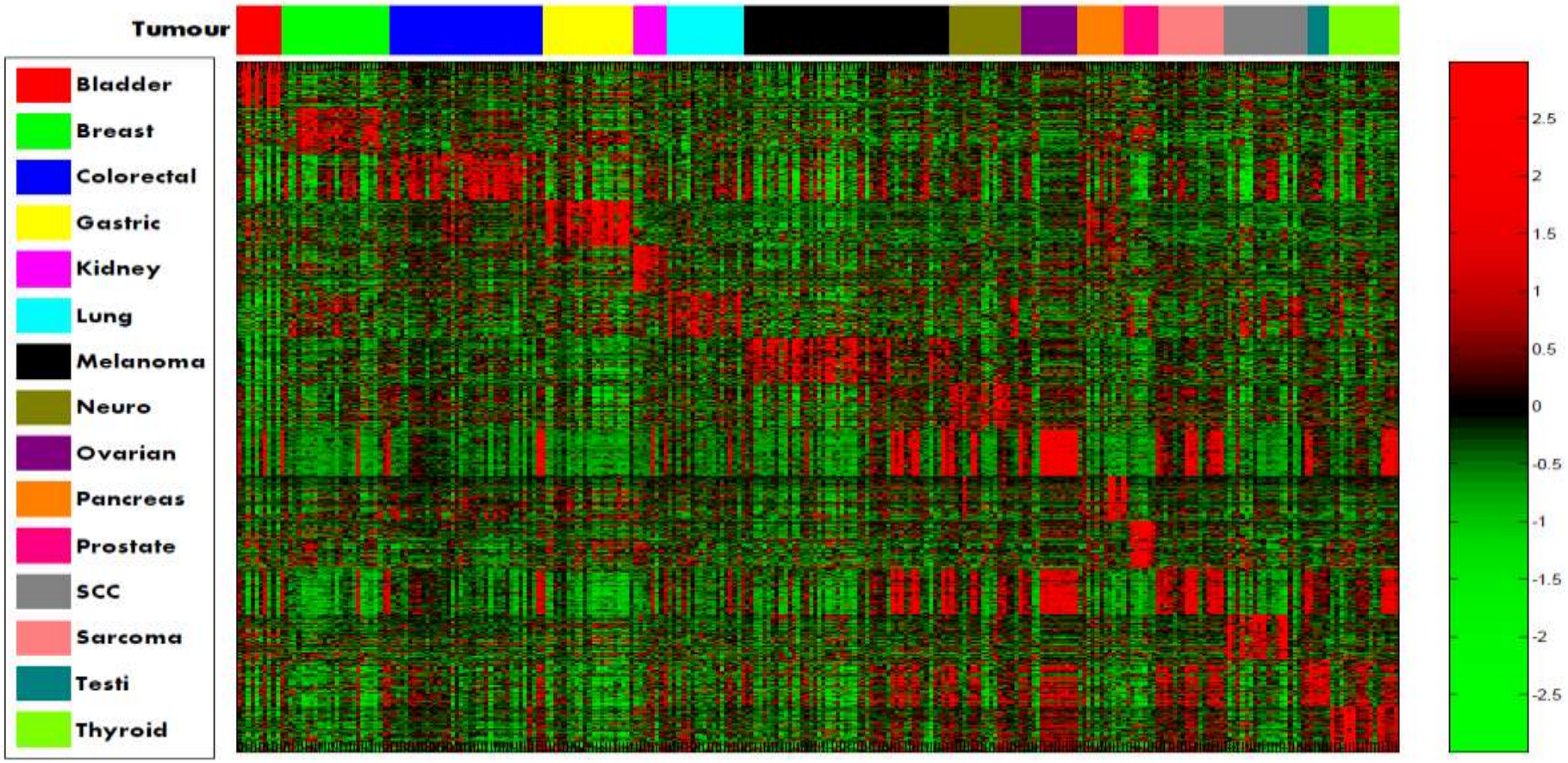
Richard Tothill



- 250 primary and metastatic tumours (fresh frozen)
- 16 histological types
- 32 histological and molecular subtypes

Tothill et al (2005) Cancer Research

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 1 : Classifier accuracies and specificities.

Tumour Class	N	Correct call with first prediction	Correct call within first two predictions	Correct call within first three predictions	Specificity
Overall Average		**83%**	**89%**	**93%**	**98.5%**
Bladder	13	85%	92%	92%	99.7%
Breast	56	79%	93%	96%	96.7%
Colorectal	48	85%	92%	96%	96.4%
Gastric	26	81%	85%	89%	99.2%
Kidney	14	93%	100%	100%	99.7%
Lung	30	83%	97%	97%	97.6%
Melanoma	50	78%	84%	88%	98.8%
Neuroendocrine	18	83%	89%	89%	98.7%
Ovarian	22	77%	96%	100%	98.4%
Pancreas	18	72%	78%	83%	97.7%
Prostate	19	89%	95%	95%	99.7%
Sarcoma	29	76%	83%	86%	96.6%
SCC	27	93%	93%	93%	99.2%
Testi	8	88%	88%	88%	99.7%
Thyroid	21	91%	91%	100%	100%

Application of classifier to CUP cases

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

Disease presentation and histology	Differential at initial presentation	Array prediction and outcome
P00459: 40-y-old male nonsmoker, no previous history. Supraclavicular and mediastinal lympadenopathy, lymphangitis of lung, right upper lobe mass, and liver metastases. Poorly differentiated adenocarcinoma.	Clinical picture most consistent with lung but uncertain in a young nonsmoker.	Lung (70). Minor response to platinum/ gemcitabine, stable disease for 3 mo on gefitinib and progressive disease with docetaxel.
P01328: 52-y-old female, no previous history. Extensive abdominal tumor. Adenocarcinoma.	Ovary, gastric, and breast	Breast (100). Left supraclavicular fossa and axillary nodes developed within 2 mo of chemotherapy.
P01405: 66-y-old male nonsmoker, no previous history. Paraaortic lymphadenopathy and bone metastases. Clear cell epithelioid tumor.	Pathology review favored sarcomatoid renal cell cancer; but renal CT and MRI normal.	Renal (88).
P01698: 37-y-old female, no previous history. Pelvic mass, ascites, and left pleural effusion. Moderately differentiated adenocarcinoma with occasional signet ring features.	Pathologist thought that morphology strongly suggested nonovarian origin (e.g., gastric, colorectal, pancreas, or lung). Clinical picture consistent with ovarian cancer.	Ovarian (92). Treated with taxol/carboplatin for presumed ovarian primary. Good clinical response with normalization of CA125
P01946: 49-y-old female smoker, no previous history. Liver, bone, adrenal, and mediastinal disease. Atypical infiltrating epithelial cells forming glandlike structures.	Lung, colorectal.	Lung (60)

High confidence 11/13 cases

Tothill et al (2005) Cancer Research

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

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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.

Tsimberidou, A.M. *et al.*, *J Clin Oncol* 22, 2616-2624 (2011).

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

Stella, G.M. *et al. Hum Mutat* 32 (1), 44-50 (2010); Dova, L. *et al.*, *Clin Exp Metastasis* 24 (2), 79-86 (2007); Lipson, D. *et al.*, *Nat Med* advance online publication (2012)

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**

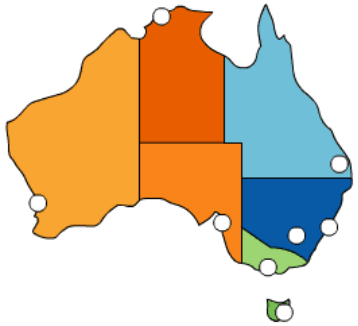
Methods

- 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 to 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?

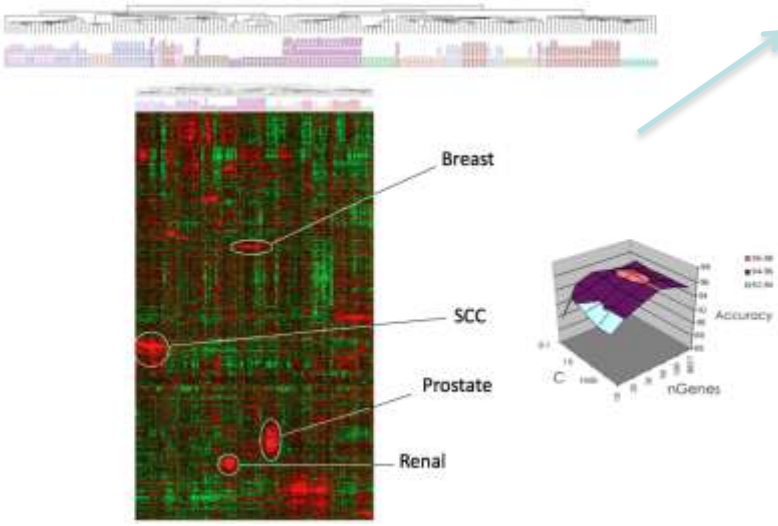
SUPER (Solving Unknown Primary cancER)



Cohort recruitment

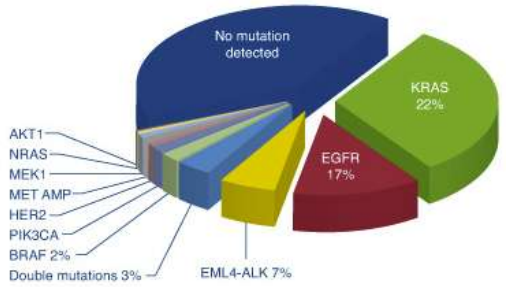
Clinical protocol development

Psychosocial studies



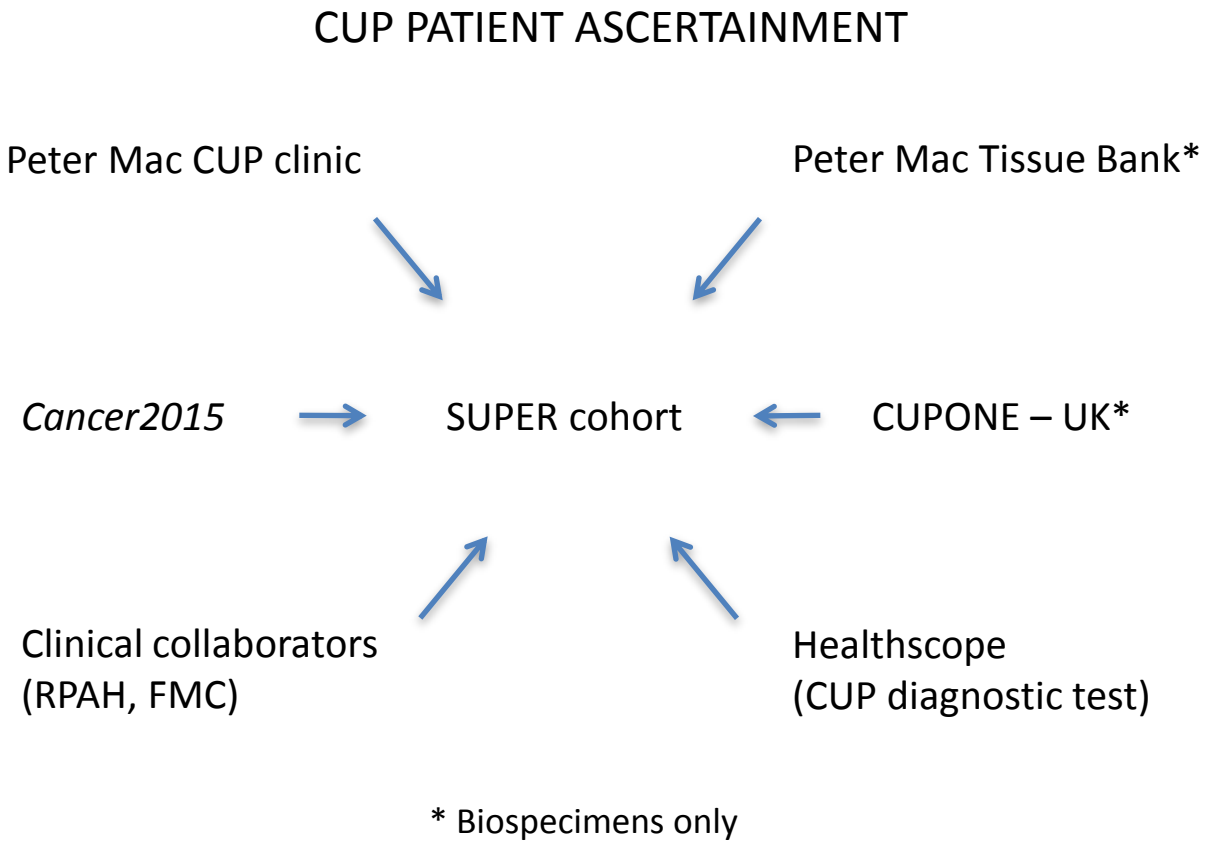
CUP diagnostic test

Tothill et al (Can Res 2005)



Actionable mutations

Cohort ascertainment



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

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