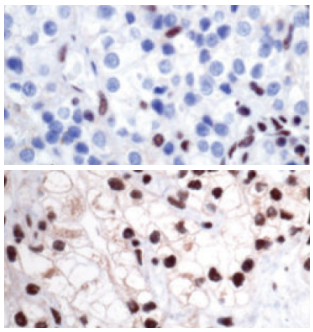
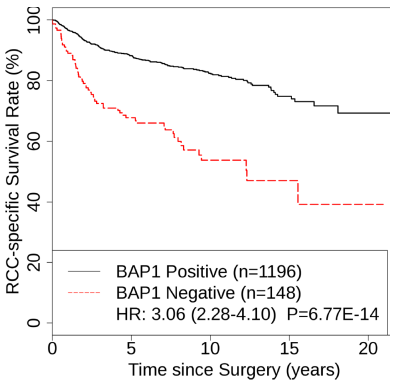
POUNDBURY CANCER INSTITUTE
for Personalised Medicine

BAP-1 test identifies the most aggressive RCC forms
even among those with low-risk parameters

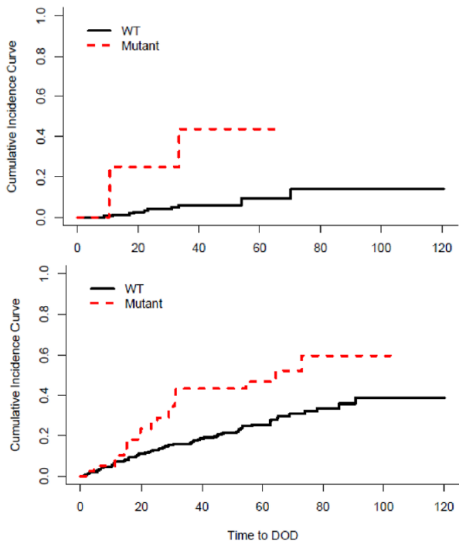


BAP-1 (BRCA-associated protein 1) is a tumour suppressor protein lost or mutated in renal cell carcinoma (RCC). Its gene is located close to VHL (von Hippel-Lindau), PBRM1 (Polybromo 1) and SETD2 (SET domain-containing protein 2) in a region of Ch3p that is frequently deleted in Renal cell carcinoma (RCC). BAP-1 loss or mutation is linked to tumour progression and aggressive behaviour in a number of cancers such as uveal melanoma, mesothelioma, "spitzoid" cutaneous melanoma and squamous cell carcinoma of the lung. Mutation or loss of BAP-1 is responsible for a small number of breast cancer, meningioma, cholangiocarcinoma and leiomyosarcoma.

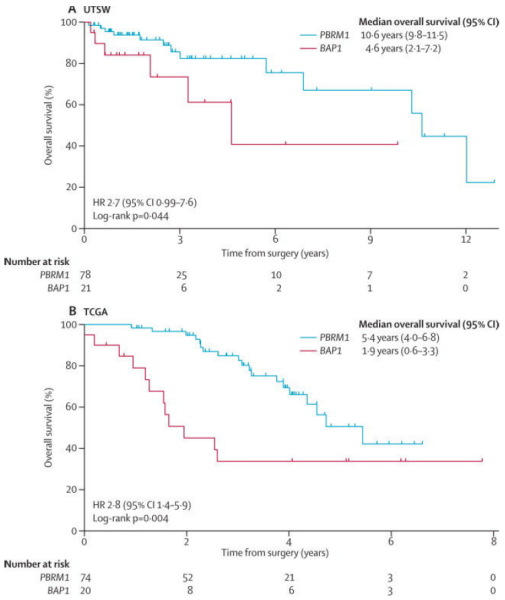
BAP-1 loss of function correlates almost invariably with loss of protein expression within tumour tissue therefore determination of BAP-1 expression has become the test of choice for BAP-1 function. Numerous studies have validated the association between prognosis and BAP-1 in RCC and the use of protein expression as surrogate marker of BAP-1 function [1, 2, 3, 4].



BAP-1$^{(-)}$ patients have significantly (HR 3.06; 95% CI 2.28-4.10; p $6.77x10^{-14}$) higher risk of RCC-specific death compared to BAP-1$^{(+)}$ patients (from 1).



Competing risk cumulative incidence curves for the MSKCC cohort (189 patients, top) and TCGA cohort (446 patients, bottom) that shows that patients with BAP-1 mutation have worse prognosis (from 2).



Overall survival for UTSW (A) and TCGA (B) cohorts stratified for mutations of BAP-1 and PBRM-1 (from 3).

We recommend the use of BAP-1 protein expression for all RCC patients, especially those categorised as "low-risk". This can be done quickly and cheaply on a preoperative sample (needle biopsy) or on a sample from surgical resection. We determine BAP-1 expression by immunohistochemistry using formalin-fixed paraffin-embedded tumour tissue. We can provide this test with rapid turn around time (24h from receipt of the sample). Urgent (same day) results are possible by prior arrangement.

Renal cancer

In 2013 11,870 new patients were diagnosed with renal cancer cases in the UK. The incidence is increasing by 1.25% per year. Although early stage renal cancer has good survival (83% at 5 years for stage I and II), the overall 5 years survival is poor (55%). This means that this year in the UK there will be over 5,500 deaths from renal cancer.

Risk stratification is of value to the patient and is useful to select the best therapeutic approach, including type and extent of surgery. There are a number of prognostic nomograms and algorithms used for stratifying the risk of relapse. Among the best known are the Memorial Sloan-Kettering Cancer Center (MSKCC) nomogram, the UCLA Integrated Staging System (UISS), the Mayo Clinic Stage Size Grade and Necrosis (SSIGN) score, the Karakiewicz nomogram and the Leibovich score.

These nomograms use a number of clinical parameters at presentation such as age, sex, local symptoms and performance status as well as data derived from the pathology assessment of the tumour, such as histological subtype, pathological stage, nuclear grade, tumour size, necrosis, vascular invasion and regional lymph node status. For this reason, risk stratification is normally done after surgery.

Despite the overall effectiveness of risk stratification, a proportion of ''low risk'' tumours do exhibit aggressive clinical behaviour and 30% of patients with low risk clear cell renal cell carcinoma will relapse after surgery. This has led to the development of molecular algorithms for risk stratification using RNA extracted from tumour tissue. For instance the Cleveland Clinic recurrence score, that uses quantitative PCR of 16 specific mRNA species[5] and the Zurich algorhythm, that uses 5 miRNA species[6]. Recent studies have showing how determination of BAP-1 status is an important biomarker for RCC patients and that this can be done quickly and effectively with little burden on resources.

At present, risk stratification after surgery is primarily used to determine the most appropriate follow-up, since there is no recognised role for adjuvant therapy in renal cancer. This is likely to change due to the emergence of a number of effective targeted treatments (for instance VEGF inhibitors such as bevacizumabm sorafenib, sunitinib, pazopanib and axitinib or mTOR inhibitors such as temsirolimus and everolimus), as well as chemo-immunotherapy and checkpoint inhibitors. Risk stratification, therefore, will prove useful also to select patients enrolment in clinical trials for new molecules and it is envisaged that a classification of RCC based on driver mutation will facilitate this.

Poundbury Cancer Institute has a remit to promote adoption of personalised medicine. We believe that BAP-1 should be a biomarker offered as standard to all RCC patients. We have managed to make this test available for £12.50. If possible we would like to keep a record of any treatment decisions made as a consequence of BAP-1 status we provide. While we would like to retain the surplus FFPE tissue to facilitate EQA and the development of future tests, our offer is not dependant on this.

1. Joseph RW et al. Loss of BAP1 protein expression is an independent marker of poor prognosis in patients with low risk clear cell renal cell carcinoma. *Cancer* 2014 **120**(7): 1059-67
2. Hakimi AA et al. Adverse outcomes in clear cell renal cell carcinoma with mutations of 3p21 epigenetic regulators BAP1 and SETD2: a report by MSKCC and the KIRC TCGA Research Network. *Clin Cancer Res* 2013, **19**(12): 3259–3267
3. Kaput P et al. Effects on survival of *BAP1* and *PBRM1* mutations in sporadic clear-cell renal cell carcinoma: a retrospective analysis with independent validation. *Lancet Oncol*. 2013, **14**(2): 159-67
4. Gossage L et al. Clinical and pathological impact of VHL, PBRM1, BAP1, SETD2, KDM6A, and JARID1c in clear cell renal cell carcinoma. *Genes Chromosomes Cancer*. 2014, **53**(1): 38-51
5. Rini B et al. A 16-gene assay to predict recurrence after surgery in localised renal cell carcinoma: development and validation studies. *Lancet Oncol*. 2015 **16**(6): 676-85
6. Christinat Y and Krek W. Integrated genomic analysis identifies subclasses and prognosis signatures of kidney cancer. *Oncotarget* 2015, **6**(12): 10521-31.

Note for the pathologist

BAP-1 is a useful prognostic marker for uveal melanoma and epithelioid cutaneous melanoma. Used in conjunction with p16, it is also useful in the differential diagnosis of mesothelioma vs benign mesothelial proliferation. It should also be considered as part of the investigations required to assess ''atypical'' spitzoid proliferation and dysplastic naevus.