Effects on survival of *BAP1* and *PBRM1* mutations in sporadic clear-cell renal-cell carcinoma: a retrospective analysis with independent validation

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Summary

Background Clear-cell renal-cell carcinomas display divergent clinical behaviours. However, the molecular genetic events driving these behaviours are unknown. We discovered that *BAP1* is mutated in about 15% of clear-cell renal-cell carcinoma, and that *BAP1* and *PBRM1* mutations are largely mutually exclusive. The aim of this study was to investigate the clinicopathological significance of these molecular subtypes and to determine whether patients with *BAP1*-mutant and *PBRM1*-mutant tumours had different overall survival.

Methods In this retrospective analysis, we assessed 145 patients with primary clear-cell renal-cell carcinoma and defined *PBRM1* and *BAP1* mutation status from the University of Texas Southwestern Medical Center (UTSW), TX, USA, between 1998 and 2011. We classified patients into those with *BAP1*-mutant tumours and those with tumours exclusively mutated for *PBRM1* (*PBRM1*-mutant). We used a second independent cohort (n=327) from The Cancer Genome Atlas (TCGA) for validation. In both cohorts, more than 80% of patients had localised or locoregional disease at presentation. Overall both cohorts were similar, although the TCGA had more patients with metastatic and higher-grade disease, and more TCGA patients presented before molecularly targeted therapies became available.

Findings The median overall survival in the UTSW cohort was significantly shorter for patients with *BAP1*-mutant tumours (4·6 years; 95% CI 2·1–7·2), than for patients with *PBRM1*-mutant tumours (10·6 years; 9·8–11·5), corresponding to a HR of 2·7 (95% CI 0·99–7·6, p=0·044). Median overall survival in the TCGA cohort was 1·9 years (95% CI 0·6–3·3) for patients with *BAP1*-mutant tumours and 5·4 years (4·0–6·8) for those with *PBRM1*-mutant tumours. A HR similar to the UTSW cohort was noted in the TCGA cohort (2·8; 95% CI 1·4–5·9; p=0·004). Patients with mutations in both *BAP1* and *PBRM1*, although a minority (three in UTSW cohort and four in TCGA cohort), had the worst overall survival (median 2·1 years, 95% CI 0·3–3·8, for the UTSW cohort, and 0·2 years, 0·0–1·2, for the TCGA cohort).

Interpretation Our findings identify mutation-defined subtypes of clear-cell renal-cell carcinoma with distinct clinical outcomes, a high-risk *BAP1*-mutant group and a favourable *PBRM1*-mutant group. These data establish the basis for a molecular genetic classification of clear-cell renal-cell carcinoma that could influence treatment decisions in the future. The existence of different molecular subtypes with disparate outcomes should be considered in the design and assessment of clinical studies.

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Introduction

More than 60 000 new cases and 13 000 deaths from tumours of the kidney and renal pelvis were expected in the USA in 2012.[1] About 70% of renal-cell carcinomas present with localised disease and about 30% of patients who undergo surgery with curative intent may experience a recurrence.[2] Several variables that influence outcome have emerged, including TNM stage, tumour size, Fuhrman grade, and necrosis.[3–6] In the metastatic setting, patients can be stratified on the basis of clinical and laboratory parameters.[7] Heng and colleagues[8] stratified patients with metastatic renal-cell carcinoma into favourable (median overall survival, not reached), intermediate (median overall survival, 27 months), and poor (median overall survival, 8·8 months) risk groups using the variables: time from diagnosis

to treatment, Karnofsky performance status, haemoglobin, corrected calcium, neutrophil count, and platelet count. However, what determinants in the tumour account for the different behaviours is unknown.

Different behaviours may be driven by different mutations. Clear-cell renal-cell carcinoma, which accounts for 70–80% of all renal-cell carcinomas,[2] is characterised by inactivation of the von Hippel-Lindau gene (*VHL*).[9] Additionally, truncating mutations in *PBRM1* (polybromo 1), a gene encoding an SWI/SNF chromatin-remodelling complex component, are found in 41% of clear-cell renal-cell carcinomas.[10] Although mutated at a substantially lower frequency, other genes implicated in clear-cell renal-cell carcinoma are *SETD2*, *KDM6A* (UTX), and *KDM5C* (JARID1C).[10–12] However,

whether any of these mutations affect outcomes is unknown.

Recently, we reported that the gene *BAP1* (BRCA1 associated protein-1) was mutated in about 15% of clear-cell renal-cell carcinoma.[13] Interestingly, *BAP1* and *PBRM1* mutations in tumours are largely mutually exclusive.[13] Additionally, whereas tumours with *BAP1* mutations are typically of high grade, tumours exclusively mutated for *PBRM1* tend to be of lower grade.[13] These results led us to hypothesise that *BAP1*-mutated tumours could be associated with worse outcomes than *PBRM1*-mutated tumours. We did a retrospective analysis comparing overall survival of patients with *BAP1*-mutated versus *PBRM1*-mutated tumours and validated the results using a second independent cohort.

Methods

Study population
We did a retrospective analysis with an initial study cohort that included 176 patients who underwent resection of a clear-cell renal-cell carcinoma at the University of Texas Southwestern Medical Center (UTSW), TX, USA, between 1998 and 2011, and whose tumours were genotyped for *BAP1* and *PBRM1*.[13] To be included in the mutation analyses, patients had to have 70% or more tumour cellularity on sections flanking a fresh-frozen specimen to be assessed for genetic analyses.[13] For this study, patients were excluded if samples were derived from metastases (four cases) or follow-up information was missing (27 cases). The remaining 145 patients were included in the UTSW cohort. These studies were done under a protocol approved by the UTSW institutional review board, in accordance to the Health Insurance Portability and Accountability Act guidelines, and patients provided written informed consent.

Data collection
We obtained clinical and pathological data retrospectively from medical records and electronic databases, and we entered the data into a standardised database. We surveyed the Social Security Death Index (SSDI) for dates of death.

A pathologist (PK) masked to the mutation status centrally reviewed archived haematoxylin and eosin stained slides for 123 patients. For the remaining 22 patients, we obtained available data from pathology reports. We determined tumour histology and grade according to 2004 WHO criteria[14] and the Fuhrman grading system.[15] Presence of any tumour spindle cells reminiscent of sarcoma was sufficient to consider the tumour as exhibiting sarcomatoid dedifferentiation. We assigned rhabdoid histology if there were foci of high-grade malignant cells with abundant eosinophilic cytoplasm, globular eosinophilic paranuclear inclusion bodies, large eccentric vesicular nuclei, and prominent

nucleoli occupying at least one field (10× objective). We defined tumour necrosis as microscopic coagulative necrosis. We staged patients using radiographic reports and postoperative pathological data and reassigned according to the American Joint Committee on Cancer 2010 TNM classification.[16] Generally, patients were followed up postoperatively with physical examination, laboratory studies, chest imaging, as well as abdominal and pelvic CT scans twice a year for the first 2 years and annually thereafter for 5 years.

We compared findings from the UTSW cohort with a publically available, open-access, dataset of clear-cell renal-cell carcinoma from The Cancer Genome Atlas (TCGA) (July 16, 2012, update). In the TCGA, tumour necrosis was ascertained on the basis of sections flanking a small specimen to be used for molecular studies. To qualify, histological slides had to have more than 50% tumour nuclei. In four instances, the clinical stage did not correspond to the TNM information provided and was excluded from analyses.

Procedures
We assessed the canonical mTORC1 markers, phosphorylated S6 ribosomal protein (S235/236), and phosphorylated 4E-BP1 (T37/46), to evaluate for mTORC1 activation.[13]

We obtained RNA-Seq information from the TCGA Data Portal, which was available for 308 clear-cell renal-cell carcinomas annotated with mutations. RNA-Seq data were aligned with MapSplice and quantified and normalised with the reads per kb of exon model per million mapped reads (RPKM) method by the TCGA. We derived gene expression signatures by comparing tumours with mutations in *BAP1* (n=20; or *PBRM1* [n=66]) to the rest using unpaired *t* tests adjusted for the group variances and a Benjamini and Hochberg false discovery rate (FDR) correction.[17] 19 of 20 532 genes did not provide valid p values and were eliminated from the analyses. We assessed the significance of the gene expression signatures by comparing the number of genes identified to the number found in groups made up of random tumours of the same size (n=20 or 66) using a one-sample *t* test. We analysed pathways using Ingenuity Pathway Analysis software.

Unless otherwise indicated, we classified tumours into *BAP1*-mutant tumours and tumours exclusively mutated for *PBRM1* (referred to as *PBRM1*-mutant in this report) on the basis of previously reported somatic (non-silent) mutations.[13]

Statistical analysis
We determined associations between a mutation group and patient or tumour characteristics using a Fisher's exact test (for categorical variables) or a Student's *t* test (for continuous variables). We computed overall survival from the date of nephrectomy to the date of death from any cause. Patients alive at the end of the

study period were censored at the date of last follow-up or the last date the patient was known to be alive, whichever was longer. We assessed overall survival using Kaplan-Meier estimates and did comparisons using the log-rank test. We obtained hazard ratios (HRs) from Cox regression analyses. We report time-to-event results with HR, 95% CI for the HR, and the log-rank p value. Unless indicated, p values are two-sided without adjustment for multiple comparisons. To assess whether a mutation was independently associated with outcome in the cohort of patients with BAP1-mutated or PBRM1-mutated tumours, we included variables that were associated with overall survival at the 0·20 level in multivariate Cox regression models after a backwards conditional method, in which we removed the variable with the worst p value one at a time until all variables left in the model were significant at the 0·05 level. We excluded pN from the model in both cohorts because it had missing data for half of the patients. We used grade 2 as the reference since there were only two grade 1 patients in each cohort. All variables were treated as categorical except for age. We did the statistical analyses with SAS 9.2 for the multivariate Cox regression analyses and SPSS Statistics 17.0 for the rest.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. PK, SPL, and JB had full access to all the data in the study and the corresponding author had final responsibility for the decision to submit for publication.

Results

The median age of patients from the UTSW cohort was 62 years (IQR 54–70) and there was a male predominance (table 1). The median tumour size was 5·7 cm (4·1–8·7). 76 (52%) of the 145 tumours were of high grade (Fuhrman grade 3 and 4). Seven (11%) of the 63 patients who underwent regional lymph node dissection had nodal metastases and 13 (9%) of 145 patients had distant metastases at the time of surgery.

We investigated a second, independent, cohort of patients with clear-cell renal-cell carcinoma from TCGA. At the time of our analyses, mutation data were available for 327 patients, which represent the cohort analysed. The median age was 61 years (IQR 52–71), with a male predominance (table 1). 187 (58%) of the 325 tumours were of high grade. 12 (8%) of 159 patients who underwent regional lymph node resection had nodal metastases and 52 (16%) of 327 had distant metastases at the time of surgery. Overall, the TCGA and UTSW cohorts were similar, although more patients presented with higher grade and metastases in the TCGA cohort.

Of the patients in the UTSW cohort, 21 had BAP1-mutated tumours, including three with mutations in both BAP1 and PBRM1 (table 2). 78 patients had tumours exclusively mutated for PBRM1. VHL mutations were present in 15 (71%) of 21 BAP1-mutant tumours and 68 (87%) of 78 PBRM1-mutant tumours. A comparison of patients with BAP1-mutated versus PBRM1-mutated tumours showed that patients with BAP1-mutated tumours were more likely to present with aggressive features including higher grade, sarcomatoid and rhabdoid histology, tumour necrosis, and mTORC1 activation (p<0·05 for all; table 2).

Of the patients in the TCGA cohort, 20 had BAP1-mutant tumours (including four tumours with mutations in both BAP1 and PBRM1) and 74 tumours with mutations exclusively in PBRM1 (table 2).

	UTSW (n=145)	TCGA (n=327)
Age		
Median (IQR)	62 (54–70)	61 (52–71)
Race		
White	103/132 (78%)	288/322 (89%)
Hispanic	16/132 (12%)	15/322 (5%)
African American	9/132 (7%)	11/322 (3%)
Indian	3/132 (2%)	0/322 (0%)
Asian	0/132 (0%)	8/322 (2%)
Native American	1/132 (<1%)	0/322 (0%)
Sex		
Female	65/145 (45%)	118/327 (36%)
Male	80/145 (55%)	209/327 (64%)
Fuhrman grade		
1	2/145 (1%)	5/325 (2%)
2	67/145 (46%)	133/325 (41%)
3	54/145 (37%)	136/325 (42%)
4	22/145 (15%)	51/325 (16%)
pT		
T1	67/145 (46%)	149/327 (46%)
T2	22/145 (15%)	41/327 (12%)
T3	50/145 (34%)	131/327 (40%)
T4	6/145 (4%)	6/327 (2%)
pN		
0	56/63 (89%)	147/159 (92%)
1	7/63 (11%)	12/159 (8%)
M		
0	132/145 (91%)	275/327 (84%)
1	13/145 (9%)	52/327 (16%)
Stage (clinical)		
I	65/145 (45%)	145/323 (45%)
II	18/145 (12%)	31/323 (10%)
III	45/145 (31%)	93/323 (29%)
IV	17/145 (12%)	54/323 (17%)

Data are number (%) unless otherwise stated. UTSW=University of Texas Southwestern Medical Center. TCGA=The Cancer Genome Atlas.

Table 1: **Patient and tumour characteristics**

Consistent with the results in the UTSW cohort, *BAP1*-mutated tumours showed a trend towards higher grade (p=0·095) and *BAP1* mutation was associated with necrosis (p=0·038). Additionally, *BAP1*-mutated tumours were more likely to have advanced pT (p=0·011) and clinical stage (p=0·003; table 2). Overall, *BAP1*-mutant tumours were uniformly associated with indicators of poor outcome.

In the UTSW cohort, patients with *BAP1*-mutant tumours had a median overall survival of 4·6 years (95% CI 2·1–7·2), which was substantially shorter than that of patients with *PBRM1*-mutated tumours, whose median overall survival was 10·6 years (9·8–11·5). The differences in overall survival corresponded to a HR of

See **Online** for appendix

2·7 (95% CI 0·99–7·6; p=0·044; figure 1). As in the UTSW cohort, in the TGCA cohort, patients with *BAP1*-mutated tumours had a significantly higher probability of death (HR 2·8, 1·4–5·9; p=0·004; figure 1). Median overall survival in the TCGA cohort for patients with *BAP1*-mutant tumours was 1·9 years (95% CI 0·6–3·3) and for those with *PBRM1*-mutant tumours, 5·4 years (4·0–6·8). Although the median overall survival values differed between the TCGA and UTSW cohorts, possibly indicating differences in the patient population and the availability of targeted therapies at the time of presentation (see appendix and discussion), the HR in both cohorts were almost identical (figure 1). These data show that *BAP1*-mutated tumours are associated with significantly worse overall survival than *PBRM1*-mutated tumours.

To assess how representative the cohorts of patients with *BAP1*-mutated and *PBRM1*-mutated tumours were, we did univariate Cox regression analyses. As expected, in both the UTSW and TCGA cohorts, pN, M, stage, grade, and necrosis were all associated with overall survival (appendix). Race, on the other hand, was not associated with overall survival in either cohort (appendix).

We did multivariate Cox regression analyses with all variables that reached 0·20 significance in univariate analyses in each cohort respectively (appendix), except for pN, which had missing data for half of the patients. A backwards elimination process to identify the best fit model showed that M and grade were independently associated with overall survival in the UTSW cohort (appendix). Other known predictors of outcome were not recovered, possibly owing to the small sample size. In addition to M and grade, mutations in *BAP1* and *PBRM1* were independently associated with overall survival in the TCGA cohort (HR 2·3; 95% CI 1·03–5·1; p=0·041; table 3).

Our previous studies with a small number of samples suggested that *BAP1*-mutated and *PBRM1*-mutated tumours have different gene expression signatures.[13] To test this notion further, we analysed gene expression signatures from RNA-Seq data of the TCGA cohort (available for 308 [94%] of the 327 samples with mutation information). We asked whether *BAP1*-mutant tumours could be distinguished from the rest. A comparison of gene expression between *BAP1*-mutated tumours (n=20) and the rest identified 3250 genes that were deregulated in the *BAP1*-mutant group after a false-discovery rate (FDR) correction (q<0·05). To ascertain the significance of these deregulated genes, we looked at how many genes would distinguish a group of the same size chosen randomly. When 20 tumours were chosen at random, only 115 genes distinguished this group from the rest. This analysis was repeated twice, and the numbers that distinguished these arbitrary groups were 63 and 120, respectively (table 4). The differences in the number of

	UTSW			TCGA		
	PBRM1 (n=78)	BAP1 (n=21)	p value	PBRM1 (n=74)	BAP1 (n=20)	p value
pT			0·28			0·011
1	33/78 (42%)	7/21 (33%)		40/74 (54%)	4/20 (20%)	
2	10/78 (13%)	6/21 (29%)		11/74 (15%)	3/20 (15%)	
3	33/78 (42%)	7/21 (33%)		23/74 (31%)	13/20 (65%)	
4	2/78 (3%)	1/21 (5%)		0/74 (0%)	0/20 (0%)	
pN			0·33			0·59
0	33/36 (92%)	11/14 (79%)		29/31 (94%)	13/15 (87%)	
1	3/36 (8%)	3/14 (21%)		2/31 (6%)	2/15 (13%)	
M			1·00			0·081
0	70/78 (90%)	19/21 (90%)		65/74 (88%)	14/20 (70%)	
1	8/78 (10%)	2/21 (10%)		9/74 (12%)	6/20 (30%)	
Stage (clinical)			0·76			0·003
I	32/78 (41%)	7/21 (33%)		40/73 (55%)	3/20 (15%)	
II	10/78 (13%)	4/21 (19%)		9/73 (12%)	2/20 (10%)	
III	28/78 (36%)	7/21 (33%)		16/73 (22%)	9/20 (45%)	
IV	8/78 (10%)	3/21 (14%)		8/73 (11%)	6/20 (30%)	
Fuhrman grade			<0·0001			0·095
1	2/78 (3%)	0/21 (0%)		2/74 (3%)	0/19 (0%)	
2	41/78 (53%)	3/21 (14%)		32/74 (43%)	4/19 (21%)	
3	31/78 (40%)	9/21 (43%)		32/74 (43%)	9/19 (47%)	
4	4/78 (5%)	9/21 (43%)		8/74 (11%)	6/19 (32%)	
Necrosis			0·029			0·038
No	47/64 (73%)	9/20 (45%)		65/74 (88%)	13/20 (65%)	
Yes	17/64 (27%)	11/20 (55%)		9/74 (12%)	7/20 (35%)	
Sarcomatoid			0·0010			..
No	76/78 (97%)	15/21 (71%)		..	..	
Yes	2/78 (3%)	6/21 (29%)		..	..	
Rhabdoid			0·00034			..
No	74/78 (95%)	13/21 (62%)		..	..	
Yes	4/78 (5%)	8/21 (38%)		..	..	
Phospho-S6	1·2 (0·2)	3·8 (0·6)	0·0010	..	..	..
Phospho-4E-BP1	1·3 (0·2)	2·2 (0·4)	0·029	..	..	..

Data are number (%) or mean (SE), for immunohistochemistry. UTSW=University of Texas Southwestern Medical Center. TCGA=The Cancer Genome Atlas.

Table 2: Characterisation of *BAP1*-mutant tumours and tumours exclusively mutated for *PBRM1* in the UTSW and TCGA cohorts

genes identified between the *BAP1*-mutant group (3250 genes) and the groups of random tumours was highly significant (p<0·0001; table 4). These data show that *BAP1*-mutated tumours are associated with a characteristic gene expression signature.

A comparison of *PBRM1*-mutant tumours (n=66) to the rest revealed 2235 genes that distinguished these tumours at an FDR q lower than 0·05 (table 4). When compared with three groups of 66 tumours selected at random, the difference in the number of distinguishing genes (2235 *vs* 0, 0, and 3) was highly significant (p<0·0001; table 4). Thus, *PBRM1*-mutated tumours are also associated with a characteristic gene expression signature. Notably, most genes that made up both signatures were downregulated in the mutant tumours. 2600 (80%) of the 3250 genes that made up the *BAP1* signature were downregulated in *BAP1*-mutant tumours and, 1910 (85%) of the 2235 genes that made up the *PBRM1* signature were downregulated in *PBRM1*-mutant tumours.

The number of genes in common between the *BAP1* and *PBRM1* signatures was 369 (figure 2). However, the overlap expected at random was 381. Thus, the signatures were non-overlapping, which indicated aberrations in different pathways (figure 2). The appendix lists genes in the *BAP1* and *PBRM1* signatures that most clearly distinguished these groups. Broadly, *BAP1*-mutant tumours were associated with changes in the expression of genes implicated in growth-factor signalling, whereas *PBRM1*-mutant tumours showed expression changes in genes implicated in the cytoskeleton and tissue architecture.

Finally, although the number of double-mutant tumours was very small (three in the UTSW cohort and four in the TCGA cohort), histological evaluation of the UTSW cohort had suggested that these tumours were associated with rhabdoid features and might be particularly aggressive.[13] In keeping with these results, Kaplan-Meier estimates of the UTSW cohort showed that double-mutant tumours were associated with the worst outcomes (HR 5·3, 95% CI 1·2–22·9; p=0·012; appendix). This was also the case for the TCGA cohort (HR 10·4, 3·2–33·6, p<0·0001; figure 3). We also included in our analyses tumours for which mutations in *BAP1* or *PBRM1* were not identified (figure 3, appendix). However, this group is likely to be heterogeneous and made up by more than one molecular genetic subtype.

Discussion

Our findings show that clear-cell renal-cell carcinoma can be subclassified into at least two biologically and clinically distinct entities: *BAP1*-mutant and *PBRM1*-mutant tumours. These tumours were associated with distinct gene expression signatures, and therefore different biology, and *BAP1*-mutant tumours displayed pathological features suggestive of aggressive disease.
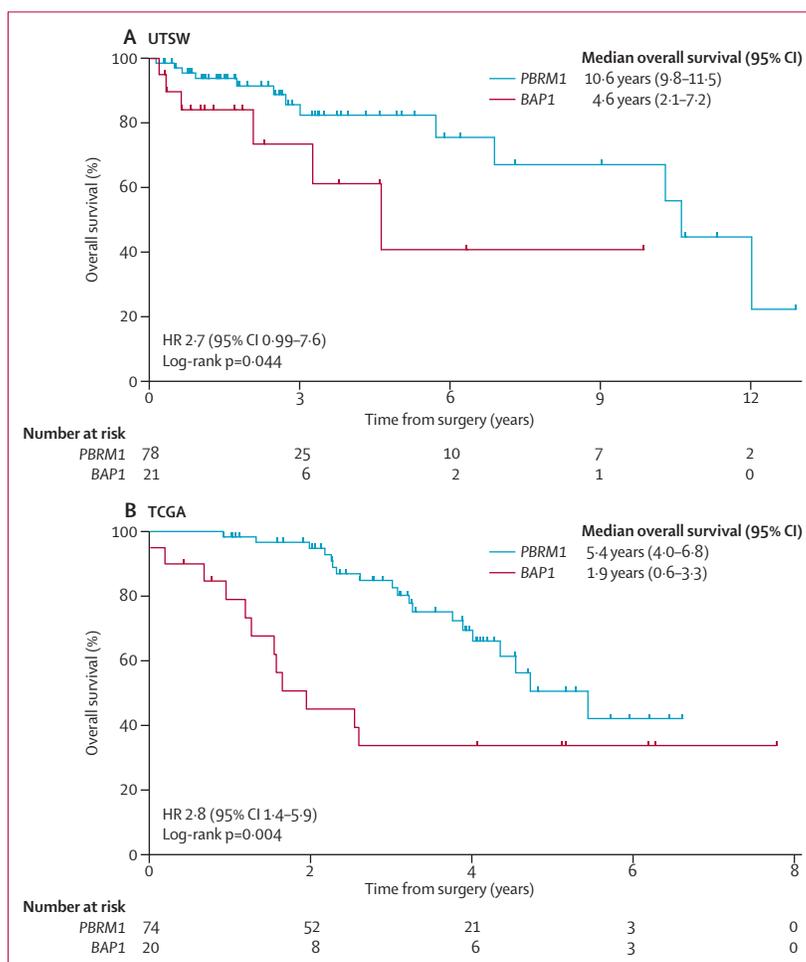
Figure 1: **Kaplan-Meier curves of overall survival**
HR=hazard ratio. (A) UTSW cohort and (B) TCGA cohort.

|	HR (95% CI)	p value
Mutation group		0·041
PBRM1	1·00 (reference)	
BAP1	2·3 (1·03–5·1)	
M		0·023
No	1·00 (reference)	
Yes	2·7 (1·1–6·2)	
Grade		0·018
1	NA	
2	1·00 (reference)	
3	0·8 (0·3–2·0)	
4	3·3 (1·3–8·6)	

NA=not applicable.

Table 3: **Multivariate analysis of time to death for patients with mutated tumours in the TCGA cohort**

BAP1-mutant tumours were associated with significantly worse overall survival than *PBRM1*-mutant tumours. This difference corresponded to a HR of 2·7
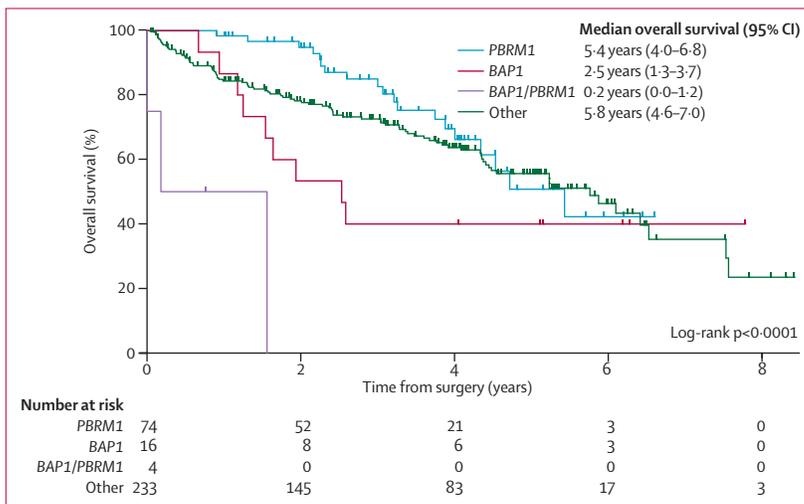
	BAP1-mutated (n=20) *vs* the rest (n=288)	p value	PBRM1-mutated (n=66) *vs* the rest (n=242)	p value
BAP1 (n=20)	3250	<0·0001	..	..
PBRM1 (n=66)	..	..	2235	<0·0001
Random 1	115	..	0	..
Random 2	63	..	0	..
Random 3	120	..	3	..

Number of genes that distinguish *BAP1*-mutant and *PBRM1*-mutant groups when compared with the rest *vs* control groups of the same size (n=20 or n=66) made up of random tumours. p values are for comparisons between the mutated groups and the random groups.

Table 4: Gene expression signatures in *BAP1*-mutant and *PBRM1*-mutant tumours
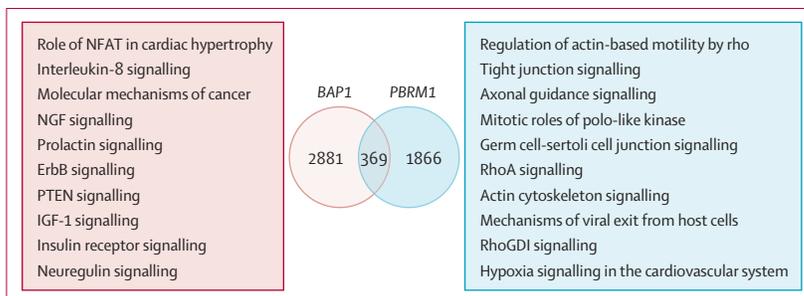
Figure 2: Pathway analysis for the *BAP1* and *PBRM1* gene signatures
Venn diagrams with number of genes that characterise *BAP1*-mutant and *PBRM1*-mutant tumours, as well as top ten deregulated pathways in each group.

Figure 3: Kaplan-Meier curves of overall survival for the indicated groups from the TCGA cohort

Fuhrman grade than *PBRM1*-mutant tumours. The main determinant of Fuhrman grading in everyday practice is nucleolar prominence,[19] which by itself is associated with survival.[20] The nucleolus is the site within the cell where ribosomes are synthesised. Ribosomes are necessary for mRNA translation, and both ribosome biogenesis and mRNA translation are regulated by mTORC1.[21] Furthermore, nucleolar size has been linked to mTORC1 activity.[22,23] In renal-cell carcinoma, a correlation was found between Fuhrman grading and S6 phosphorylation,[24] a marker of mTORC1 activation. Interestingly, *BAP1* mutation is linked to mTORC1 activation (although this association is likely to be indirect),[13] which may contribute to explain the connection between *BAP1* mutation, high Fuhrman grade, and outcome. Notably, in uveal melanoma, where *BAP1* is also somatically mutated, *BAP1* mutations were present in most metastasising but in only a few non-metastasising tumours.[25]

Clear-cell renal-cell carcinoma is characterised by *VHL* mutations, but *VHL* inactivation alone is insufficient for tumour initiation.[26,27] Both *BAP1* and *PBRM1* are two-hit tumour suppressor genes and they are located on chromosome 3p (where *VHL* is found), in a region that is deleted in the majority of sporadic clear-cell renal-cell carcinoma.[14,28] We speculate that, in many instances, the development of clear-cell renal-cell carcinoma is initiated by a focal mutation in *VHL*, followed by a 3p deletion. 3p loss may eliminate *VHL* gene function and would leave cells with just one copy of *BAP1* and *PBRM1*. Mutation of the remaining *BAP1* or *PBRM1* allele may initiate tumorigenesis, resulting in tumours of different aggressiveness, depending on which gene is mutated. Thus, tumour aggressiveness may be established early on during the process of tumorigenesis.

Possibly accounting for macroscopic and microscopic differences within tumours, significant genetic heterogeneity has been reported in primary clear-cell renal-cell carcinoma.[29] According to their prevalence, mutations can be classified into ubiquitous, shared, and private. Ubiquitous mutations are present in all tumour cells and include initiating events. We postulate that mutations in *BAP1* and *PBRM1* (as well as *VHL*) represent ubiquitous, truncal, driver events of tumour development. Therefore, pathways deregulated by these mutations are ideal drug targets. Thus, the discovery of *BAP1* and *PBRM1* mutations in clear-cell renal-cell carcinoma may pave the way for the next generation of targeted therapies. Given the particularly poor outcomes of *BAP1*-mutant tumours, identifying vulnerabilities resulting from BAP1 loss is particularly important. Once candidate drugs are found, their assessment may be helped by the availability of tumourgraft mouse models reproducing the molecular genetics and treatment responsiveness of renal-cell carcinoma in humans.[30]

(95% CI 0·99–7·6), which was almost identical in the TCGA cohort. Thus, this study establishes the foundation for the first molecular genetic classification of sporadic clear-cell renal-cell carcinoma (panel).

Why *BAP1*-mutant tumours are associated with worse survival is not understood. However, in both cohorts, when compared with *PBRM1*-mutant tumours, *BAP1* mutation in tumours was associated with coagulative necrosis, an independent predictor of outcome.[18] Additionally, *BAP1*-mutant tumours had a higher

How mutations in *BAP1* and *PBRM1* drive renal carcinogenesis is not understood. We show that *BAP1*-mutant and *PBRM1*-mutant tumours exhibit highly specific gene expression signatures that distinguish these tumours from the rest. The *BAP1* and *PBRM1* gene expression signatures are quite distinct, which is in keeping with differences in pathological features and patient outcomes. Interestingly, most genes that make up the *BAP1* signature were downregulated in *BAP1*-mutant tumours. These data raise the possibility that BAP1, which is a nuclear deubiquitinase, may act by deubiquitinating transcription factors, which in the absence of BAP1 are ubiquitinated and targeted for proteosomal-mediated degradation. Support for such a model is provided by a recent report.[31] Similarly, most genes that make up the *PBRM1* signature were downregulated in *PBRM1*-mutant tumours. PBRM1 is the chromatin targeting subunit of a nucleosome remodelling complex and we speculate that when *PBRM1* is mutated, increased levels of closed chromatin impair transcription, thereby reducing gene expression.

Other genes have been implicated in the development of clear-cell renal-cell carcinoma, such as *SETD2*, *KDM5C*, and genes of the MLL family.[10–12,32] How mutations in these genes relate to mutations in *BAP1* and *PBRM1* remains to be explored. Similarly, it is presently unknown whether these mutations define other molecular subtypes with different biology and outcomes.

This study has several limitations. First, we assessed mostly white patients, and the distribution of mutations in different patient populations remains unknown. Second, the sample size and follow-up are modest. However, for a study assessing the molecular genetics of renal cancer, two independent cohorts of 145 and 327 patients is not insubstantial, and in the TCGA cohort, median follow-up was 35 months (IQR 13–53). Additionally, mutation analyses are less susceptible to subjective calls than the more conventional immunohistochemistry studies.

More importantly, these limitations did not preclude the identification of meaningful and significant differences in overall survival. The nearly identical HR and statistically significant p values in two representative and independent patient cohorts strongly supports the notion that *BAP1*-mutant and *PBRM1*-mutant tumours are associated with distinct survival outcomes.

Third, the median overall survival for patients with either *BAP1*-mutant or *PBRM1*-mutant tumours was shorter in the TCGA cohort. However, regardless of mutation status, the overall survival for all patients was shorter for the TCGA cohort than in the UTSW cohort. Factors that could explain this difference include differences in the patient population, with higher proportions of patients with metastatic disease (16% *vs* 9%) and high grade (58% *vs* 52%) in the TCGA cohort than in the UTSW cohort. Another factor is the

availability of targeted therapies. In fact, 37% (43 of 115) of patients in the TCGA cohort died before 2006 (when molecularly targeted therapies became available) compared with only 11% (4 of 35) in the UTSW cohort (appendix). Finally, another limitation of the study is that most pathological variables did not reach significance in both cohorts. However, in both the UTSW and TCGA cohorts, *BAP1*-mutant tumours were associated with indicators of worse outcome such as necrosis (significant in both) and high grade.

Whether *BAP1* and *PBRM1* are independent predictors of outcome remains to be determined. Multivariate analysis in the subset of patients with *BAP1* or *PBRM1* mutations from the TCGA showed that mutations predicted outcome independently of other variables. Although this analysis found other known predictors of outcome (grade and M status) several established predictors failed to surface, probably because of the small sample size. Consistent with this, prognostic factors in existing nomograms were identified in substantially larger patient cohorts.[3–6] This small sample size might also explain why mutation

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Panel: Research in context

We did not do a systematic review. This work stems from discoveries made in our laboratory.[13] Through a combination of genome and exome sequencing followed by Sanger sequencing of candidate genes in a large number of clear-cell renal-cell carcinomas, we discovered that the *BAP1* gene was mutated in about 15% of clear-cell renal-cell carcinomas. We found that *BAP1* and *PBRM1* mutations in tumours were largely mutually exclusive and that BAP1 loss, but not PBRM1 loss, was associated with high tumour grade. These data suggested that the *BAP1* and *PBRM1* genes defined different subtypes of clear-cell renal-cell carcinoma that could be associated with different outcomes. In this report, we show that *BAP1*-mutant and *PBRM1*-mutant tumours are associated with distinct gene expression patterns and consequently different biology. Most importantly, we show that *BAP1*-mutant and *PBRM1*-mutant tumours are associated with disparate overall survival of patients.

Interpretation

Although the number of patients with *BAP1*-mutant and *PBRM1*-mutant tumours in each cohort was small, the HRs for overall survival were almost identical in both the UTSW and TCGA cohorts, and the log-rank p values were significant at the 0·044 and 0·004 level. These data indicate that *BAP1*-mutant and *PBRM1*-mutant tumours are associated with distinct overall survival. *BAP1*-mutant and *PBRM1*-mutant tumours showed different gene expression signatures portraying different biology and pathogenesis. This study sets the foundation for the first molecular genetic classification of sporadic clear-cell renal-cell carcinoma and paves the way for treatments tailored to the different molecular subtypes.

status was not found as an independent predictor in the UTSW cohort. Our efforts continue in the development of robust immunohistochemistry assays that accurately report on BAP1 and PBRM1 and which could facilitate their definitive assessment as independent predictors of patient survival in larger patient cohorts.

Importantly, however, this is not simply a biomarker study. Biomarkers refer to indicators of disease state that provide prognostic or predictive information. Biomarkers do not necessarily inform on the biology of the tumour, and their value is typically predicated on how much information they add to existing nomograms. Furthermore, biomarkers can represent epiphenomenological variables with poorly understood links to tumour biology. By contrast, we linked two genetic drivers of clear-cell renal-cell carcinoma to disparate outcomes. The classification we propose is based on mutations in driver genes, which are associated with distinct gene expression patterns and a different biology. The study is also not simply a classification based on gene expression, which has been reported previously.[33] Rather, the novelty of the study is in the establishment of a foundation for the first molecular classification of sporadic renal cancer based on distinct molecular genetic subtypes that are associated with different outcomes. Furthermore, these different subtypes might have different responses to treatment, and only with their recognition can drug effectiveness be properly assessed. For example, a drug could be very active against *BAP1*-deficient tumours, but since these tumours account for just 15% of all clear-cell renal-cell carcinoma, the effect would be masked in an unselected population.

Finally, although the number of patients with tumours mutant for both *BAP1* and *PBRM1* was very small in both cohorts and these tumours are rare (1–2% of all sporadic clear-cell renal-cell carcinoma), they tend to be associated with both pathological and outcome measures suggestive of greatest aggressiveness.

In conclusion, our results provide the basis for a biologically meaningful and clinically relevant molecular genetic classification of clear-cell renal-cell carcinoma that may influence strategies for improved targeted therapies.

Contributors
PK reviewed histological slides, analysed the data, reviewed the literature, and wrote the report. SP-L obtained SSDI information, managed and analysed the data, did statistical analyses, and prepared the figures. LZ and AP-J collected clinical data. AC did statistical analyses under the supervision of X-JX. WKR assisted with TCGA data access and analysis. JB conceived and supervised the study, analysed the data, and wrote the report.

Conflicts of interest
We declare that we have no conflicts of interest.

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