Supplementary Information

ATLAS investigators……...39
Supplemental Methods……..…..40
Additional inclusion criteria……..……40
Analysis of TCGA dataset……..…40
Dose reductions…….....40
Sample size calculation……40
Genomic analyses of patients' tumor samples…….....……..…41
Significantly associated co-occurring alterations……..…42
Supplemental Results…….…….…….42
Zygosity and germline characteristics……...……42
Significantly associated co-occurring alterations…….…..…43
Supplementary References……..…….43
Supplemental Figures and Tables…….……...…45
Supplementary Table S1. Most Frequent (≥10% of Patients) Treatment-Related Adverse Events of Any Grade in the Safety Population…….....45
Supplementary Table S2. Summary of the Genetic Alterations in Tumor Tissue Samples and Tumor Responses in Patients With DDR Gene Mutation……..…46
Supplementary Table S3. Comparison of Genomic Characteristics From Archival and Recently Acquired Tumor Samples……..……48
Supplementary Figure S1. Time Profile for Mean (± Standard Deviation) Trough Plasma Concentration of Rucaparib…….……..52

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Supplementary Methods

Additional inclusion criteria

Eligible patients had histologically or cytologically confirmed locally advanced unresectable (tumor, node, metastasis [TNM] staging of T4b and any N; or any T and N2-3) or metastatic transitional cell carcinoma of the urothelium (including renal pelvis, ureter, urinary bladder, or urethra); those with mixed transitional/non-transitional cell histologies were allowed. No more than one prior platinum-based chemotherapy (PBC) was permitted for advanced disease. A patient was considered to have one prior PBC if there was a change of PBC within the same treatment regimen. A combination of PBC and radiation therapy was not considered as a prior PBC regimen.

Analysis of TCGA dataset

Data from the genomic analysis of TCGA urothelial bladder carcinoma dataset reported by Robertson et al. [1] was accessed and analyzed using Cbioportal [2, 3] online and custom in-house analysis tools.

Dose reductions

Dose reductions were permitted for patients in decrements of 100 mg if a grade ≥3 or inadequately controlled grade 2 adverse event was observed.

Sample size calculation

The overall sample size was determined by considering the number of patients needed for adequate safety and activity assessment of homologous recombination deficiency (HRD)-positive patients. Based on the estimated prevalence of 60% HRD-positive patients in this population, the study was designed to enroll approximately 200 patients, 120 of whom would be HRD-positive patients. The null hypothesis for response rate based on historical data in similar patient populations was $P = 0.10$. With a total of 200 patients, the study had greater than 90% power to reject the null hypothesis at a 5% significance level if the true response rate for rucaparib was 20%.

Genomic analyses of patients' tumor samples

The data cutoff date for genomic analyses was January 14, 2020. Comprehensive genomic profiling was performed using a hybrid-capture based next-generation sequencing (NGS) approach to sequence a targeted panel of 310 cancer-related genes and to identify functionally classified gene alterations, including single nucleotide variants, short insertions/deletions, rearrangements, fusions, and copy number alterations as described in Frampton et al [4]. Zygosity and germline/somatic status of the alterations were computationally predicted based on a research–use only algorithm that has not been analytically validated [5]. An alteration in zygosity was determined by the loss of the second allele through loss of heterozygosity (LOH) at the locus of the alteration; other mechanisms that could lead to the loss of the other allele were not investigated. Genome-wide LOH was defined as the percentage of the interrogable genome with LOH and was evaluated using ~3500 single nucleotide polymorphisms as previously described [6, 7]. Genomic LOH values (for investigational use only) were reported in samples for which computationally derived tumor content was greater than 30%. Tumor mutational burden was defined as the number of mutations per megabase of DNA and was determined by measuring the number of synonymous and nonsynonymous somatic mutations occurring in the sequenced genes across 0.8–1.2

Mb genomic content as described [8]. Microsatellite status was determined by assessing the indel characteristics at 114 homopolymer repeat loci in or near the targeted gene regions analyzed as described. The genomic profiling analysis presented in this manuscript comprises one sample per patient even if multiple samples were provided; preference for inclusion was given to the most recently acquired tissue samples or those which returned genome-wide LOH results.

Significantly associated co-occurring alterations

Pairs of genes with deleterious alterations were assessed for their tendency to co-occur by quantifying how strongly the presence of one gene alteration was associated with the presence of another using the log2 odds ratio statistic. Co-occurring alterations that demonstrated a significant association were evaluated using one-sided Fisher's exact test (P <0.05) and of Benjamini-Hochberg false discovery rate correction procedure (q <0.05).

Supplementary Results

Zygosity and germline characteristics

Genomic profiling of the ATLAS samples using NGS revealed the genomic landscape of the ATLAS tumor samples by identifying single nucleotide variants, short insertions/deletions, rearrangements, fusions, and copy number alterations. Of particular interest were the deleterious DNA damage repair (DDR) gene alterations. Although zygosity and germline status were not reported for the gene rearrangement alterations, most of the identified DDR short variants were inferred to be heterozygous germline alterations. The only exceptions were two *ATM* missense alterations (one identified as homozygous and somatic, one as heterozygous and somatic), and the *RAD51C* frameshift alteration, characterized as somatic with unknown zygosity (Supplementary Table S2).

Significantly associated co-occurring alterations

Genomic analysis highlighted multiple co-occurring alterations in the ATLAS tumor samples. Co-occurring alterations that demonstrated a significant association included co-amplification of *FGF* genes (*FGF19* with *FGF3*, or *FGF4* and *FGF3* with *FGF4*); co-amplification of *CCND1* with *FGF3*, *FGF4*, or *FGF19;* co-amplification of *FGFR1* and *WHSCHL1*; co-deletion of *CDKN2A* with *CDKN2B*; and co-deletion of *CDKN2A* or *CDKN2B* with *MTAP* (Figure 4). These co-occurring copy number alterations were not expected due to the co-location within the genome of the gene pairs described.

Supplementary References

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Supplemental Figures and Tables

Supplementary Table S1 Most frequent (≥10% of patients) treatment-related adverse events of any grade in the safety population.

	Overall (*N* = 97)	
TRAE	Any grade, *n* (%)	Grade ≥3, *n* (%)
Overall	76 (78.4%)	32 (33.0)
Asthenia/fatigue	43 (44.3)	6 (6.2)
Nausea	35 (36.1)	1 (1.0)
Anemia[a]	23 (23.7)	13 (13.4)
Thrombocytopenia[b]	17 (17.5)	8 (8.2)
Vomiting	17 (17.5)	1 (1.0)
ALT/AST increased	14 (14.4)	4 (4.1)
Decreased appetite	14 (14.4)	2 (2.1)
Blood creatinine increased	12 (12.4)	0
Dysgeusia	12 (12.4)	0

ALT alanine aminotransferase; *AST* aspartate aminotransferase; *TRAE* treatment-related adverse event.
Visit cutoff date: February 20, 2020.
[a] Combined term for anemia or decreased hemoglobin.
[b] Combined term for thrombocytopenia or decreased platelets.

Supplementary Table S2 Summary of the genetic alterations in tumor tissue samples and tumor responses in patients with DDR gene mutation.[a]

Patient	Gene[a]	Deleterious alteration	Zygosity	Germline status[b]	% LOH	TMB	Best change % STL
1	*ATM*	H2872R	Homozygous	Somatic	16.8	6.3	48.9
2	*ATM*	R337C	Heterozygous	Somatic	Indeterminate[d]	2.5	–31.3
3	*BRCA1*	V409fs*3	Heterozygous	Germline	9.9	13.9	–7.8
4	*BRCA1*	E23fs*17	Heterozygous	Germline	12.0	5.0	NE[c]
5	*CHEK2*	Splice site 1096-1G>A	Heterozygous	Germline	7.2	25.2	50.0
6	*CHEK2*	I157T	Heterozygous	Germline	8.3	7.6	75.6
7	*RAD51C*	E218fs*33	Unknown[d]	Somatic	34.0	3.8	29.1
8	*BRCA1*	Rearrangement (truncating)	Not reported[e]	Not reported[e]	22.3	6.3	NE[c]
9	*BRCA2*	Rearrangement (truncating)	Not reported[e]	Not reported[e]	5.6	6.3	NE[c]
10	*PALB2*	Rearrangement (truncating)	Not reported[e]	Not reported[e]	Indeterminate[d]	16.4	10.1

DDR DNA damage repair; *LOH* loss of heterozygosity; *NE* not evaluable; *STL* sum of target lesions; *TMB* tumor mutational burden.
[a] Deleterious somatic and germline alterations in the following DDR genes are shown: *ATM, CHEK2*, *BRCA1*, *BRCA2*, *PALB2*, or *RAD51C*.
[b] Germline/somatic status was computationally predicted using a research–use only algorithm that has not been analytically validated.
[c] Not evaluable due to lack of data comparing baseline and posttreatment scans.

[d] Valid data could not be generated. [e]Zygosity and germline/somatic status were not reported for gene rearrangements.

Supplementary Table S3 Comparison of genomic characteristics from archival and recently acquired tumor samples.

Patient	Archival specimen				Recently acquired specimen[a]				Intervening therapy
	Collection date	Tissue origin	Gene	Alteration	Collection date	Tissue origin	Gene	Alteration	
11	Nov-23-2018	Bladder	CREBBP FGFR3 MLL2 CDKN2A CDKN2B MDM2 MTAP	Q923* S800fs*101 P480fs*450 loss loss amp loss	Feb-27-2019	Lymph node	CREBBP FGFR3 MLL2 CDKN2A CDKN2B MDM2 MTAP	Q923* S800fs*101 P480fs*450 loss loss amp loss	Cisplatin + gemcitabine
12	Nov-17-2017	Bladder	TERT TP53 MLL2 AKT2 AXL CCND1 CDKN2A CDKN2B FGF19 FGF3 FGF4 MTAP	promoter -124C>T H179R Q3577fs*13 amp amp amp loss loss amp amp amp loss	Sep-5-2018	Liver	TERT TP53 MLL2 RAD21 AKT2 AXL CCND1 CDKN2A CDKN2B FGF19 FGF3 FGF4 MTAP	promoter −124C>T H179R Q3577fs*13 splice site 274+1G>T amp amp amp loss loss amp amp amp loss	Cisplatin + gemcitabine
13	Dec-12-2017	Renal pelvis	HRAS MERTK TERT CDKN2A	Q61R D151V promoter −124C>T loss	Feb-8-2019	Psoas Muscle	HRAS MERTK TERT CDKN2A	Q61R D151V promoter −124C>T loss	Cisplatin + gemcitabine, atezolizumab

			CDKN2B MTAP	loss loss			CDKN2B MTAP	loss loss	
14	Jan-5-2018	Lymph node	FGFR3 PIK3CA TERT CDKN2A CDKN2B STAG2	S249C Q546R promoter −124C>T loss loss loss	Mar-11-2019	Lymph node	FGFR3 PIK3CA TERT CDKN2A CDKN2B HGF	S249C Q546R promoter −124C>T loss loss amp	Cisplatin + gemcitabine, pembrolizumab
15	Oct-13-2016	Kidney/ureter	TERT CCND1 CDKN2A CDKN2B FGF10 FGF19 FGF3 FGF4 MDM2 RICTOR WHSC1L1 ZNF703	promoter −124C>T amp loss loss amp amp amp amp amp amp amp amp	Nov-15-2018	Liver	KDM6A SF3B1 TERT CCND1 CDKN2A CDKN2B ERBB2 FGF19 FGF3 FGF4 MDM2	W449* K666N promoter −124C>T amp loss loss amp amp amp amp amp	Cisplatin + gemcitabine, pembrolizumab

amp amplification; *loss* homozygous deletion; *HCl* hydrochloride.
Red font indicates the novel deleterious gene alterations identified between the two samples.
[a] Compulsory for participation in ATLAS.

Supplementary Fig. S1 Time profile for mean (± standard deviation) trough plasma concentration of rucaparib.