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Clinical-bladder cancer

Comprehensive genomic profiling of histologic subtypes of urethral carcinomas

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Abstract

Background: Carcinoma of the urethra (UrthCa) is an uncommon Genitourinary (GU) malignancy that can progress to advanced metastatic disease.

Methods: One hundred twenty-seven metastatic UrthCa underwent hybrid capture-based comprehensive genomic profiling to evaluate all classes of genomic alterations (GA). Tumor mutational burden was determined on up to 1.1 Mbp of sequenced DNA, and microsatellite instability was determined on 114 loci. PD-L1 expression was determined by IHC (Dako 22C3).

Results: Forty-nine (39%) urothelial (UrthUC), 31 (24%) squamous (UrthSCC), 24 (19%) adenocarcinomas NOS (UrthAC), and 12 (9%) clear cell (UrthCC) were evaluated. UrthUC and UrthSCC are more common in men; UrthAC and UrthCC are more common in women. Ages were similar in all 4 groups. GA in PIK3CA were the most frequent potentially targetable GA; mTOR pathway GA in PTEN were also identified. GA in other potentially targetable genes were also identified including ERBB2 (6% in UrthUC, 3% in UrthSCC, and 12% in UrthAC), FGFR1-3 (3% in UrthSCC), BRAF (3% in UrthAC), PTCH1 (8% in UrthCC), and MET (8% in UrthCC). Possibly reflecting their higher GA/tumor status, potential for immunotherapy benefit associated with higher tumor mutational burden and PD-L1 staining levels were seen in UrthUC and UrthSCC compared to UrthAC and UrthCC. Microsatellite instability high status was absent throughout.

Conclusions: Comprehensive genomic profiling reveals GA that may be predictive of both targeted and immunotherapy benefit in patients with advanced UrthCa and that could potentially be used in future adjuvant, neoadjuvant, and metastatic disease trials. © 2020 Published by Elsevier Inc.

Keywords: Urethral cancer; Cancer genetics; Targeted cancer therapy

Carcinomas of the urethra (UrthCa) are rare tumors that can arise in both women and men [1−3]. There are 4 major subtypes of UrthCa: urothelial carcinomas (UrthUC), squamous cell carcinomas (UrthSCC), adenocarcinomas NOS (not otherwise specified) (UrthAC), and clear cell carcinomas (UrthCC) [3]. Other uncommon malignancies can arise in the urethra including melanomas [1]. When compared with urothelial carcinomas of the urinary bladder, UrthCa are exceedingly rare [4]. Primary UrthCa is significantly more common in men than women with one study

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finding the disease in 1075 men and 540 women with an annual age-adjusted incidence rate of 4.3 per million and 1.5 per million, respectively [4]. The male preponderance is restricted to the UrthUC and UrthSCC with UrthAC and UrthCC more common in women than in men [3]. Surgery has been the main primary therapy for UrthCa with postoperative radiation treatment offered to patients whose primary tumors are larger or extend near or are present at the surgical margins [5,6]. Although the prognosis is generally favorable for patients whose tumors are successfully resected, local relapses and the development of systemic disease are well documented [7,8]. For patients with postsurgical relapsed clinically advanced disease and patients who present with advanced-stage disease, platinum drug-based chemotherapy has been the systemic treatment of choice [9−11]. Variable responsiveness of the metastatic UrthCa to systemic chemotherapy have been described [12]. For stage IV patients whose tumors become refractory to primary chemotherapy, other treatment options have been limited [9−13].

Given the well-documented impact of employing a personalized approach to patients with metastatic carcinoma originating in the urinary bladder, including both targeted and immunotherapies, recent interest has emerged as to whether a similar strategy could impact the clinical outcome of patients suffering from clinically advanced UrthCa [10]. The following comprehensive genomic profiling (CGP) study of 127 patients with metastatic and clinically advanced UrthCa describes the genomic alterations (GA), microsatellite instability (MSI) status, and tumor mutation burden associated with each tumor subtype and the potential clinical impact of these results on the selection of therapies for these patients.

1. Methods

Approval for this study, including a waiver of informed consent and a HIPAA waiver of authorization, was obtained from the Western Institutional Review Board (Protocol No. 20152817). DNA was extracted from FFPE tissue obtained from 127 clinically advanced cases of urethral carcinomas including 49 UrthUC, 31 UrthSCC, 34 UrthAC, and 12 UrthCC that had progressed to inoperable recurrent disease or clinically evident metastatic disease at the time of sequencing. When the exact clinical presentation was provided at the time samples were received for sequencing, 100% of UrthCa were metastatic. CGP was performed in a Clinical Laboratory Improvement Amendments (CLIA)-certified, College of American Pathologists (CAP)-accredited laboratory. Central review by a board-certified pathologist was performed for all cases including evaluation of H&E-stained slide to confirm pathologic diagnosis and review of the accompanying pathology report (IHC results were available for some but not all UrthCA cases). Of note, a single block was submitted for review in all cases. All samples forwarded for DNA extraction contained a minimum of 20% tumor nuclear area, compared with the benign nuclear area. In brief, ≥50 ng DNAs was extracted from 40 μm of tumor samples and assayed by CGP using adaptor-ligation and hybrid capture performed for all coding exons of from 287 (version 1) to 324 (version 3) cancer-related genes plus select introns from 19 (version 1) to 28 (version 3) genes frequently rearranged in cancer. Sequencing of captured libraries was performed using the Illumina HiSeq technology to a mean exon coverage depth of >600X, and resultant sequences were analyzed for base substitutions, insertions, deletions, copy number alterations (focal amplifications and homozygous deletions), and select gene fusions, as previously described [14]. Known confirmed somatic alterations deposited in the Catalog of Somatic Mutations in Cancer (COSMIC v62) were highlighted as biologically significant [15]. All inactivating events (i.e., truncations and deletions) in known tumor suppressor genes were also called significant. To maximize mutation-detection accuracy (sensitivity and specificity) in impure clinical specimens, the test was previously optimized and validated to detect base substitutions at a ≥5% mutant allele frequency (MAF), indels with a ≥10% mutant allele frequency with ≥99% accuracy, and fusions occurring within baited introns/exons with >99% sensitivity [14]. Tumor mutational burden (TMB) was determined on 0.9 to 1.1 megabases (Mb) of sequenced DNA for each case based on the number of somatic base substitution or indel alterations per Mb after filtering to remove known somatic and deleterious mutations as previously described [16]. Assessment of MSI was performed from DNA sequencing across the coding regions of >300 genes. To determine MSI status, optimized homopolymer repeat loci on the CGP sequencing panel were analyzed for length variability and compiled into an overall MSI score via principal components analysis. Each microsatellite locus had a repeat length of 7 to 39 bp. The next-generation sequencing-based "MSI score" was translated into categorical MSI high (MSI-H), MSI ambiguous, or microsatellite stable (MSS) by unsupervised clustering of specimens for which MSI status was previously assessed via gold standard methods (e.g., IHC) [17]. The urethral carcinoma groups were also assessed for the presence of a mutational signature indicative of a tobacco exposure/smoking history using a previously published method [18]. In this study, PD-L1 expression was determined on 5-μm tissue sections using the Dako 22C3 anti-PD-L1 antibody with low-level staining defined as 1% to 49% expression on tumor cells and high staining defined as > 50% expression on tumor cells. Statistical comparisons of genomic signatures between the 4 histologic subtypes of UrthCA were assessed using the Fisher exact method.

2. Results

The clinical and genomic features of the 127 cases of clinically advanced UrthCa are shown in Table 1. The 127

Table 1
Clinical and genomic findings in 127 cases of urethral carcinomas

	UrthUC	UrthSCC	UrthAC	UrthCC
Number of cases	49	31	34	13
Males/Females	78% M/22% F	55% M/45% F	29% M/71% F	9% M/91% F
Median age (range)	67 (44−87)	61 (40−76)	64 (40−76)	60 (33−71)
Sample used for sequencing: primary tumor	30	21	16	8
Sample used for sequencing: metastasis biopsy	19	10	18	5
GA/Tumor	6.9	10.8	5.6	4.1
Top untargetable GA	*TP53 43%*	*TP53 52%*	*TP53 79%*	*CDKN2A 23%*
	TERT 30%	*CDKNA 32%*	*CDKN2A 29%*	*MYC 23%*
	CDKN2A 28%	*TERT 21%*	*SMAD4 24%*	*TP53 23%*
	CDKN2B 22%	*MYC 16%*	*KRAS 24%*	*VEGFA 15%*
	CCND1 16%	*FAT1 14%*	*MYC 18%*	*ARID1A 15%*
Top potentially targetable GA	*PIK3CA 22%*	*PIK3CA 29%*	*PTEN 15%*	*PIK3CA 31%*
	FGFR3 12%	*EGFR 10%*	*ERBB3 12%*	*PTCH1 8%*
	BRCA2 8%	*PTEN 7%*	*PIK3CA 12%*	*TSC2 8%*
	PTEN 8%	*ERBB2 3%*	*ERBB2 12%*	*MET 8%*
	ERBB2 6%	*FGFR1 3%*	*NF1 9%*	
	TSC1 4%	*FGFR3 3%*	*BRCA2 6%*	
	BRCA1 4%	*TSC2 3%*	*EGFR 3%*	
	KIT 2%	*FGFR2 3%*	*BRAF 3%*	
MSI high	0%	0%	0%	0%
Median TMB	5.2	4.3	4.3	5.0
TMB ≥10/20 mutations/Mb	22%/10%	23%/6%	9%/0%	9%/0%
PD-L1 low/high positive (tumor immune cells)	0%/0%	50%/20%	29%/0%	NA
HPV16/18	14%	23%	0%	0%

GA = genomic alterations; MSI = microsatellite instability; TMB = tumor mutational burden.

UrthCa cases included 49 (39%) urothelial (UrthUC), 31 (24%) squamous (UrthSCC), 24 (19%) adenocarcinomas (UrthAC), and 12 (9%) clear cell (UrthCC). The UrthCa was more common in men in the UrthUC (78% male) and UrthSCC (55% male), whereas UrthCa cases were more common in women in the UrthAC (71% female) and UrthCC (91% female) subtype patients. The median ages were similar in all 4 UrthCa subtypes. All 127 UrthCa patients were suffering from clinically advanced disease featuring both documented metastatic disease and major local recurrences after primary treatments. At the time of DNA sequencing, the original primary tumor was used in 75 (59%) of the UrthCa cases, and a metastasis biopsy was sequenced in 52 (41%) of the cases. Metastatic biopsies were obtained from multiple sites, including lymph nodes, rectum, pelvis, soft tissue, retroperitoneum, abdomen, liver, and lung tissues.

CGP revealed a wide variety of GA in all 4 tumor subtypes which were classified into "currently untargetable GA" (no regulatory approvals or promising active clinical trials targeting the GA) and "currently potentially targetable GA" (regulatory approvals in other tumor types and active clinical trials targeting the alteration). The untargetable and targetable alterations identified in the 4 tumor types are shown in Table 1. Long tail plots of the GA in each tumor subtype are shown in Fig. 1 and detailed in Supplementary Tables 1−4). Tile plots of the GA in each separate case of the 4 subtypes of urethral carcinoma are shown in Fig. 2. The GA per tumor frequencies varied from 4.1 in UrthCC to 10.8 for UthSCC. Across all 4 tumor subtypes, GA in PIK3CA were the most frequent potentially targetable GA. mTOR pathway GA in genes such as PTEN were also frequently identified. Other notable GA in potentially targetable genes included ERBB2 GA (6% in UrthUC, 3% in UrthSCC, and 12% in UrthAC), FGFR1-3 GA (3% in UrthSCC), BRAF GA (3% in UrthAC), PTCH1 GA MET GA, and ATM GA (all 8% in UrthCC). Noteworthy comparisons of GA frequencies among the histologic subtypes included the high TP53 GA in UrthAC ($P = 0.02$), the high PTCH1, MET, TSC2, and ATM frequencies in UrthCC (NS), high PTEN-inactivating GA in UrthAC ($P < 0.05$), low PIK3CA GA in UrthAC (NS), and high EGFR GA (amplifications and fusions; no activating short variant mutations) in UrthSCC ($P = 0.01$).

Possibly reflecting their tobacco exposure associated with higher GA/tumor, the potential for efficacy of immune checkpoint inhibitor drugs (ICPI) that have been associated with higher TMB and PD-L1 staining levels were identified more often in UrthUC and UrthSCC compared to UrthAC and UrthCC. MSI-high status, however, was absent throughout. GA in genes associated with ICPI efficacy such as PBRM1 and ICPI resistance such as STK11 and KEAP1 were identified in less than 1% of cases in all UrthCa and in all subtypes. In addition, the CGP assay also identified the presence of viral DNA. HPV16 and/or HPV 18 viral DNA was identified in 14% of UrthUC and 23% of UrthSCC. HPV DNA was not identified in either the UrthAC or UrthCC tumor samples.

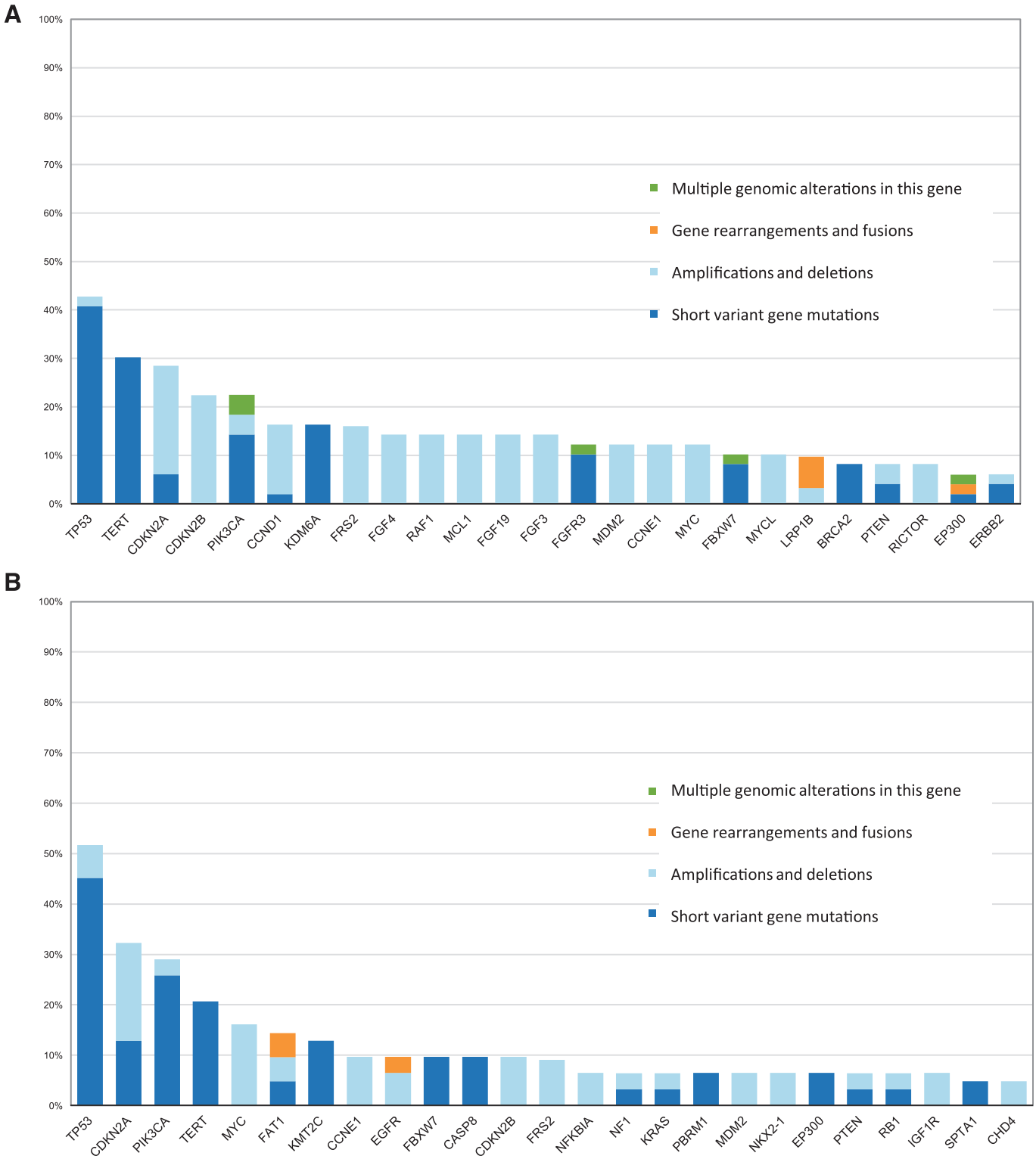
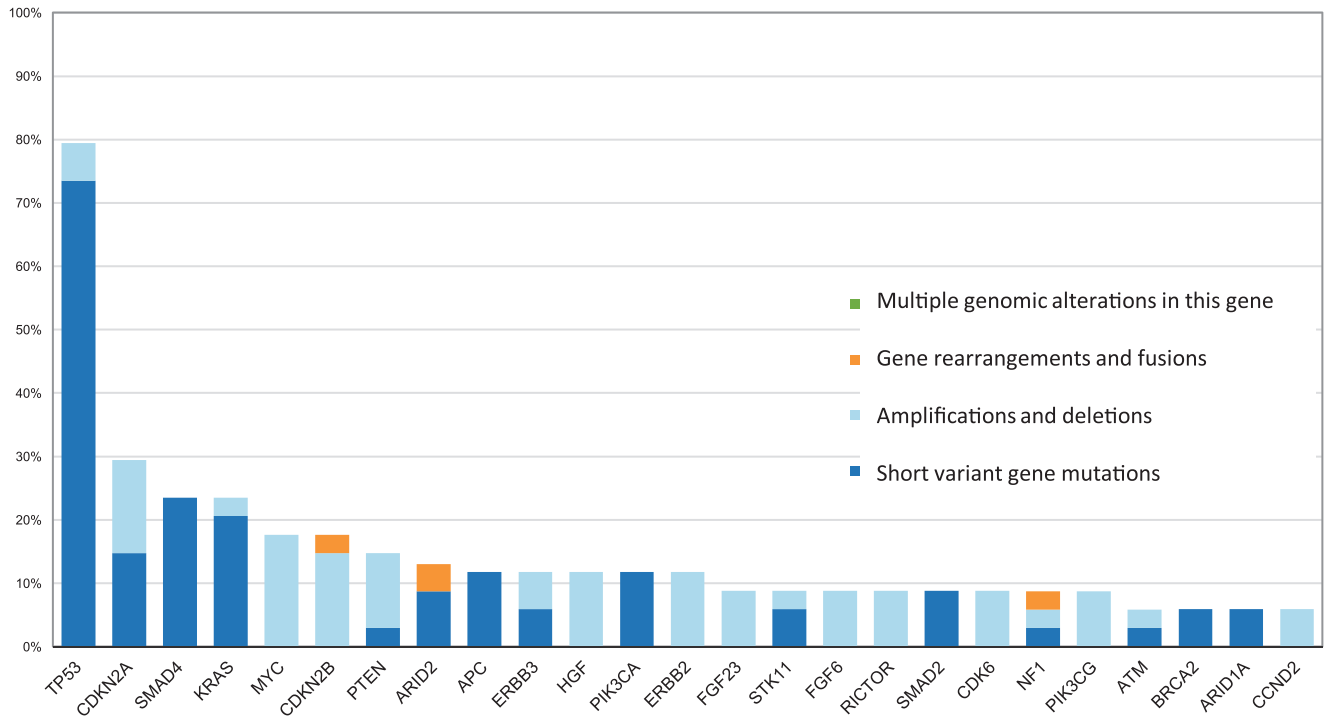


Fig. 1. Long tail plots of genomic alterations in urethral urothelial carcinoma (A), urethral squamous cell carcinoma (B), urethral adenocarcinoma NOS (C), and urethral clear cell carcinoma (D).

C



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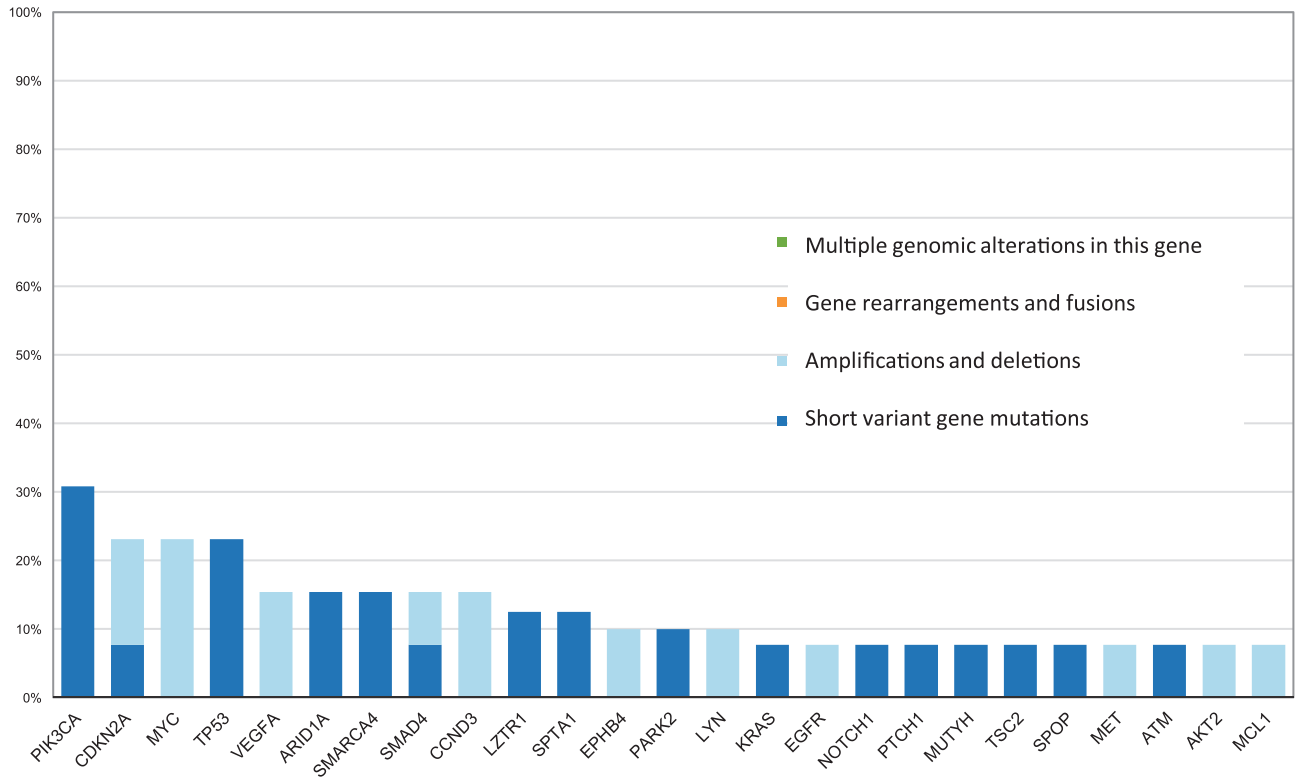


Fig. 1. Continued

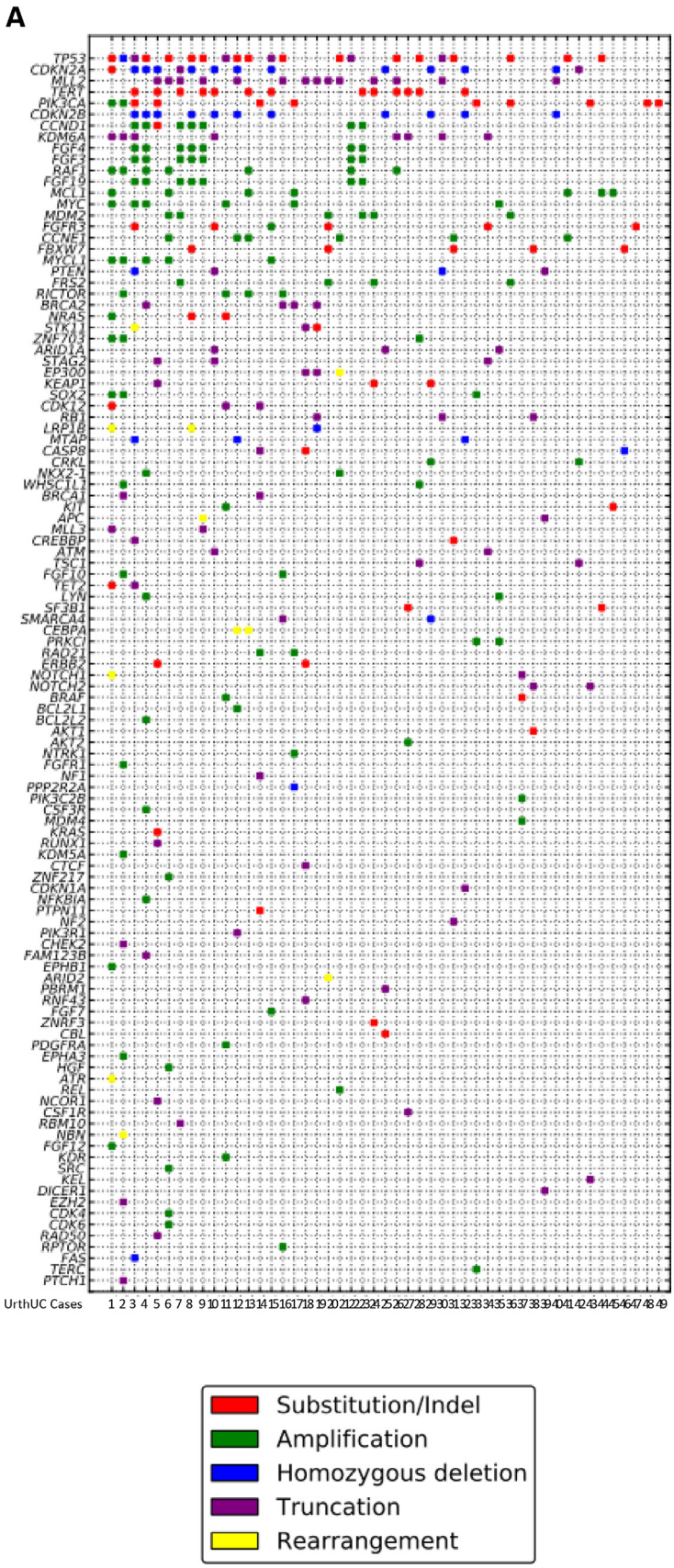
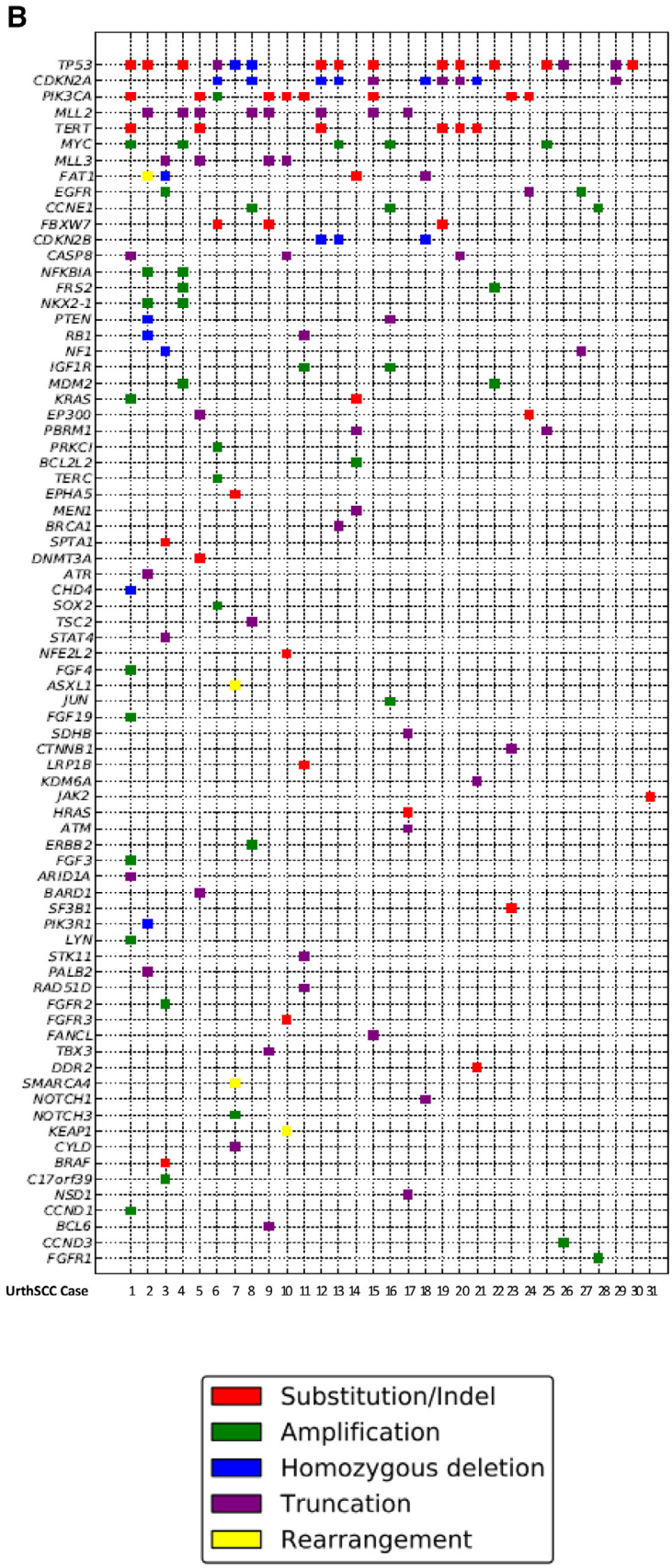


Fig. 2. (New). Tile plots of genomic alterations in urethral urothelial carcinoma (A), urethral squamous cell carcinoma (B), urethral adenocarcinoma NOS (C), and urethral clear cell carcinoma (D).



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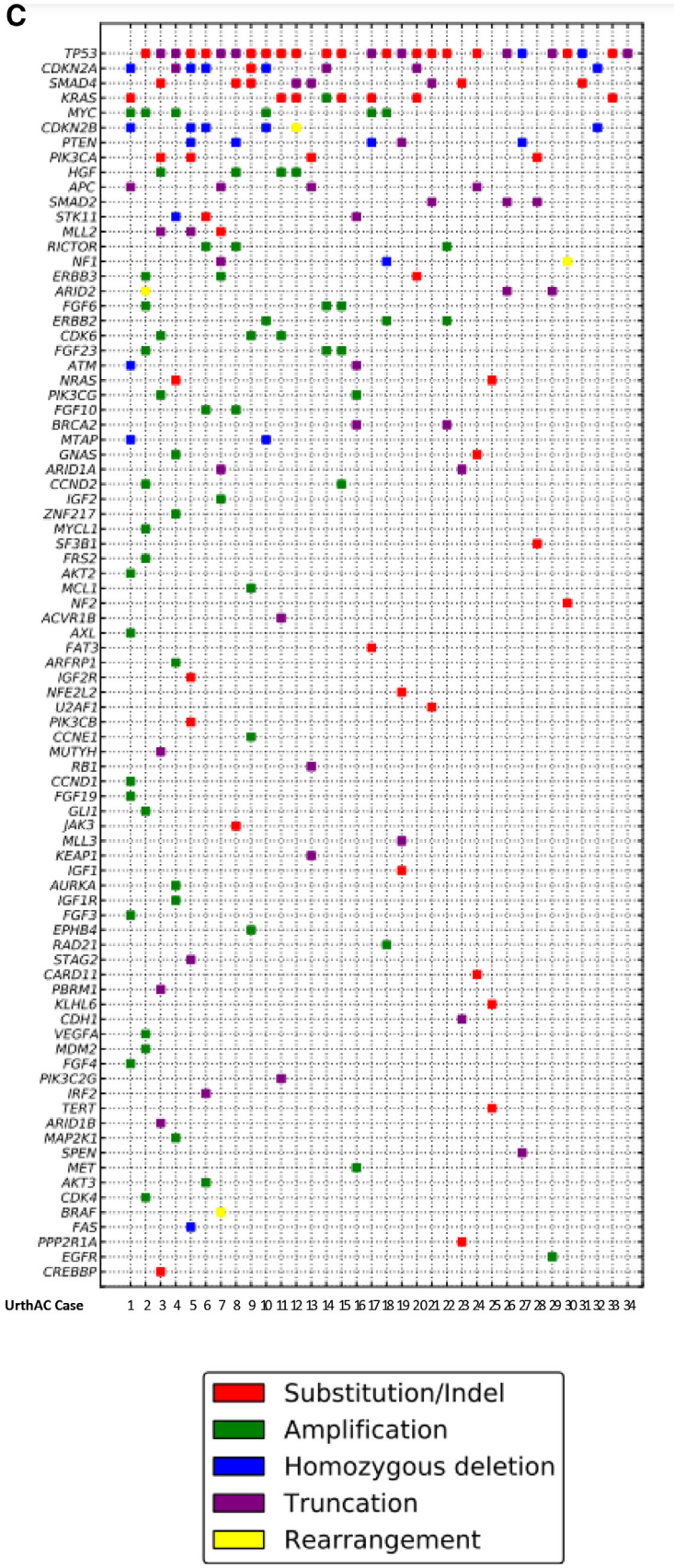

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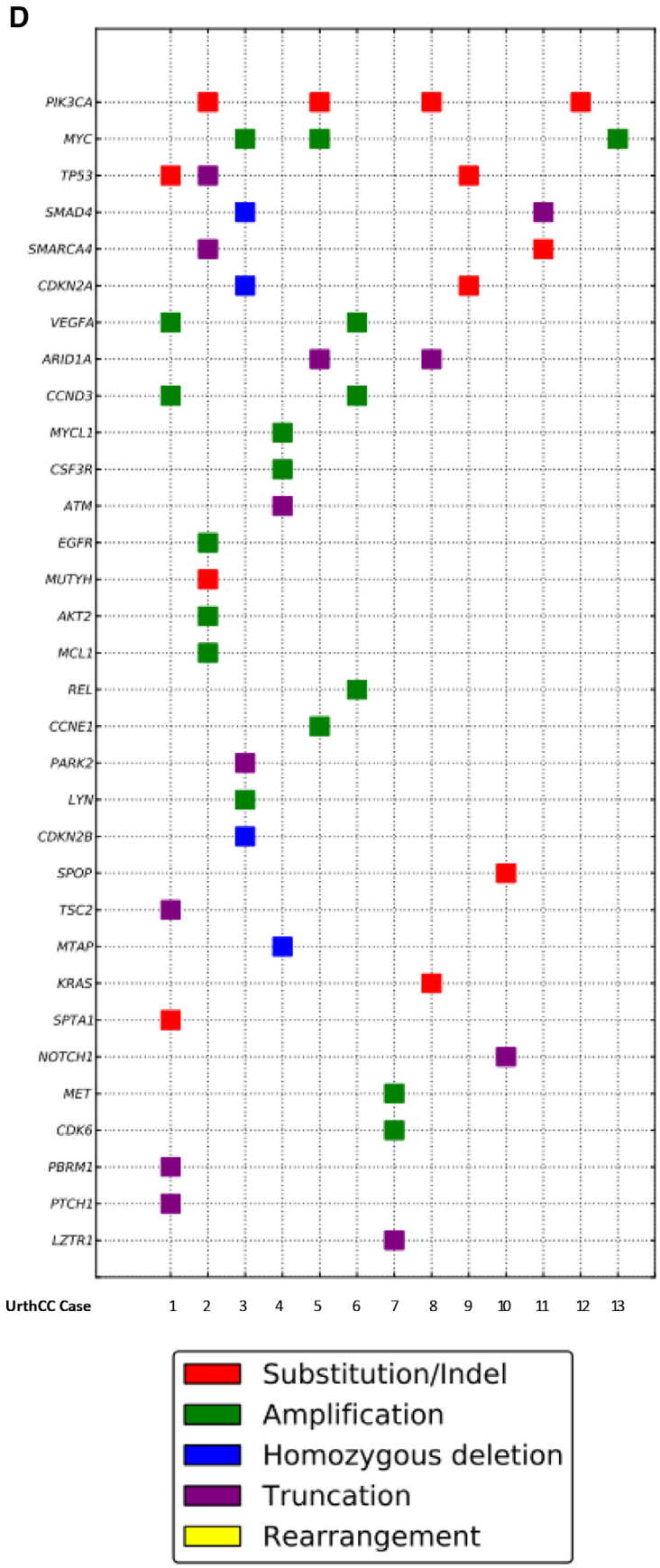

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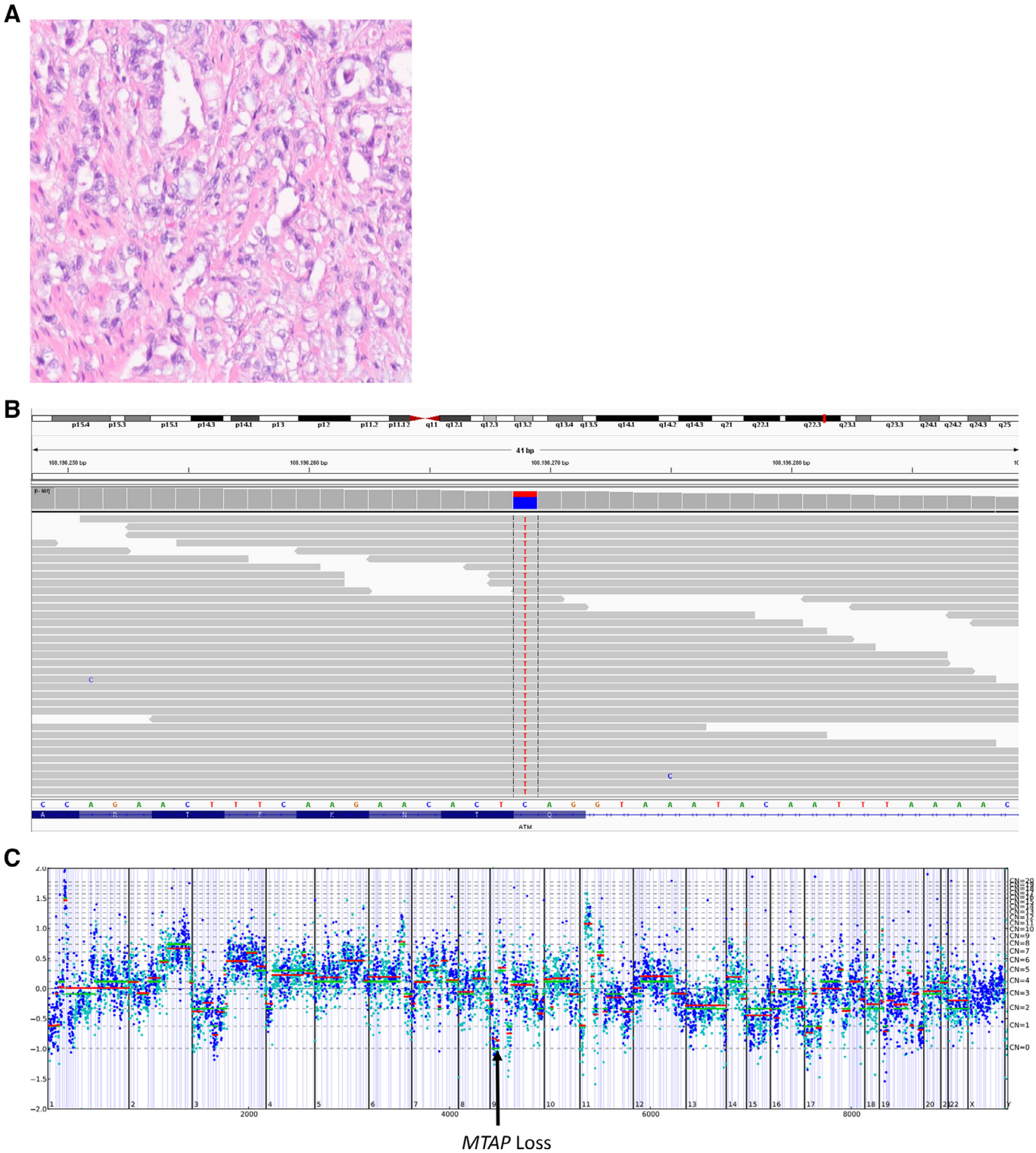

Fig. 3. (Revised). Radical surgical excision of a primary urethral clear cell carcinoma in a 72-year-old Asian female. The primary tumor encircled the entire urethra and focally extended upward to the urethral epithelium. The tumor was composed of a predominantly tubulocystic proliferation of pleomorphic tumor cells within a desmoplastic stroma (A). The tumor cells have irregular vesicular nuclei, prominent nucleoli, and a modest amount of amphophilic cytoplasm. Hobnail cells are identified. Scattered mitoses and ulceration were present. The tumor invaded the periurethral stroma and muscle (both fibromuscular and adipose tissue), the anterior vaginal wall, and the bladder wall but did not breach the bladder mucosa or the vaginal epithelium. Comprehensive genomic profiling of the primary urethral clear cell carcinoma revealed an ATM mutation (B) as demonstrated in the IGV view. ATM mutations have been linked to homologous DNA repair defects and potential responsiveness to PARP inhibitors in other tumor types such as ovarian, breast, and prostatic carcinomas. In addition, as seen in (C), there was also a copy number loss in the MTAP gene on chromosome 9p without codeletion of the CDKN2A/B genes. MTAP genomic loss is currently being developed as a genomic biomarker of tumors that may be susceptible to drugs such as PRMT-5 inhibitors that target the deranged tumor cell arginine metabolism.

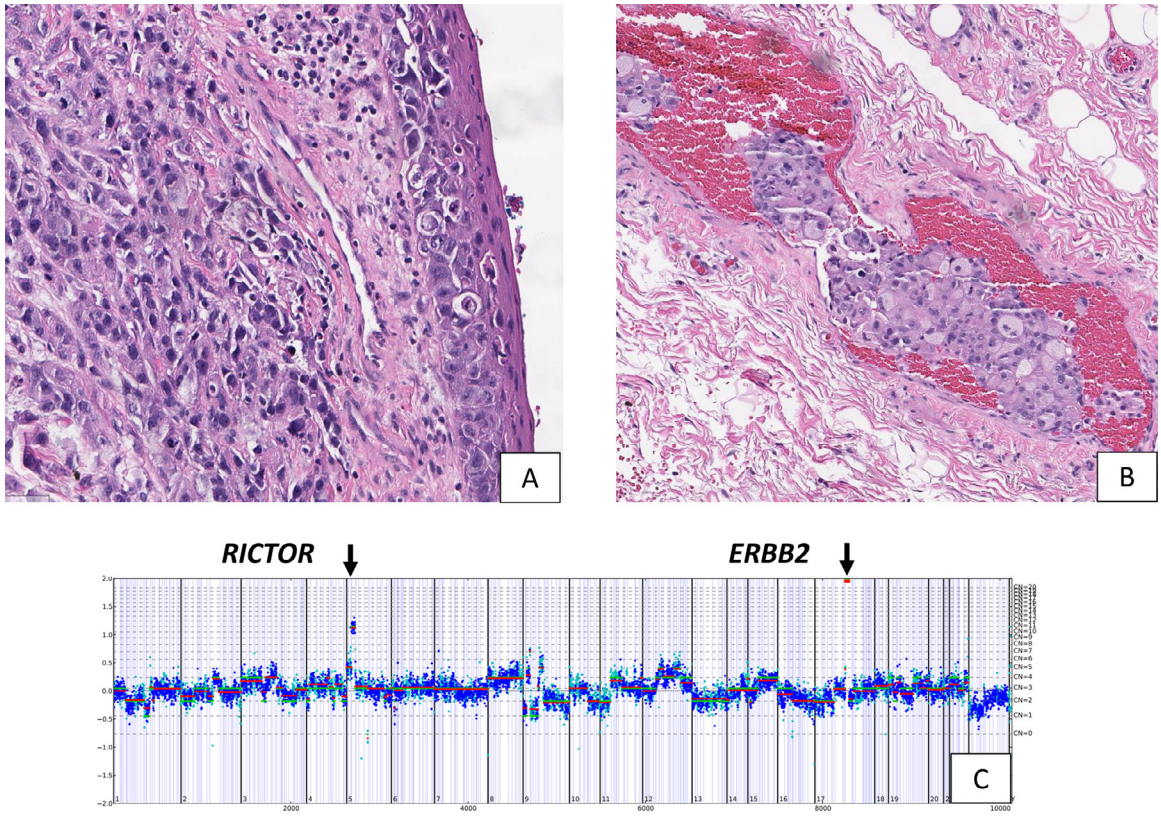

Fig. 4. A clinically advanced urethral tumor in a 74-year-old female presented with vulvovaginal Paget's Disease (A) and a deeply invasive urethral malignancy featuring extensive angioinvasion (B). This tumor was positive for CK7, GCDFP, p40, p16, CEA, p63 and negative for Mel-A and CDK5-6 on IHC staining. The final pathologic diagnosis was adenocarcinoma NOS of the urethra. On comprehensive genomic profiling, the copy number plot (C) revealed 58 copies of the ERBB2 gene along with amplification (11 copies) of the mTOR pathway gene, RICTOR, and somatic short variation mutations in BRCA2 (E1213*) and TP53 (E285K). The tumor was MSI stable and featured an intermediate TMB of 10 mutations/Mb. Major amplifications of ERBB2 such as seen in this case have been known to respond to anti-HER2-targeted therapies including both antibody therapeutics and tyrosine kinase inhibitors outside the regulatory approvals in breast and upper gastroesophageal carcinomas. Additional potential therapy options for this patient included PARP inhibitors indicated by the BRCA2 mutation and mTOR inhibitors based on the RICTOR amplification.

In Fig. 3 (revised) a primary urethral clear cell carcinoma in a 72-year-old Asian female revealed an ATM mutation. ATM mutations have been linked to homologous DNA repair defects and potential responsiveness to PARP inhibitors in other tumor types such as ovarian, breast, and prostatic carcinomas. In addition, there was also a copy number loss in the MTAP gene on chromosome 9p without codeletion of the CDKN2A/B genes. MTAP genomic loss is currently being developed as a genomic biomarker of tumors that may be susceptible to drugs such as PRMT-5 inhibitors that target the deranged tumor cell arginine metabolism. This tumor also featured amplifications of the CSF3R and MYCL1 genes, a low TMB at 5 mutations/Mb and was MS stable.

In Fig. 4, a clinically advanced urethral tumor in a 74-year-old female presented with vulvovaginal Paget's disease and a deeply invasive urethral malignancy featuring extensive angioinvasion is shown. This tumor was positive for CK7, GCDFP, p40, p16, CEA, p63 and negative for Mel-A and CDK5-6 on IHC staining. The final pathologic diagnosis was adenocarcinoma NOS of the urethra. On comprehensive genomic profiling, 58 copies of the ERBB2 gene were the major finding along with amplification (11 copies) of the mTOR pathway gene, RICTOR, and somatic short variation mutations in BRCA2 (E1213*) and TP53 (E285K). The tumor was MSI stable and featured an intermediate TMB of 10 mutations/Mb. In Fig. 5, a biopsy of a metastatic lesion in the pelvis of an 84-year-old man with a prior history of primary urethral adenocarcinoma is shown. This tumor was positive for CK7 and CK20 and negative for PSA and GATA-3 on IHC staining. Comprehensive genomic profiling revealed 2 distinct mutations in PIK3CA (C378Y and H1047R) as well as short variant mutations in SF3B1 (K700E) and SMAD2 (S464*).

3. Discussion

UrthCa is a rare malignancy that features different clinical presentations in women vs. men, multiple histologic subtypes, and varying clinical outcomes [1−3]. When UrthCa is histologically subtyped, the UrthUC and UrthSCC are significantly more frequently identified in men, whereas the UrthAc and UrthCC are more common in women [1−3]. In addition, CGP reveals that the histologic

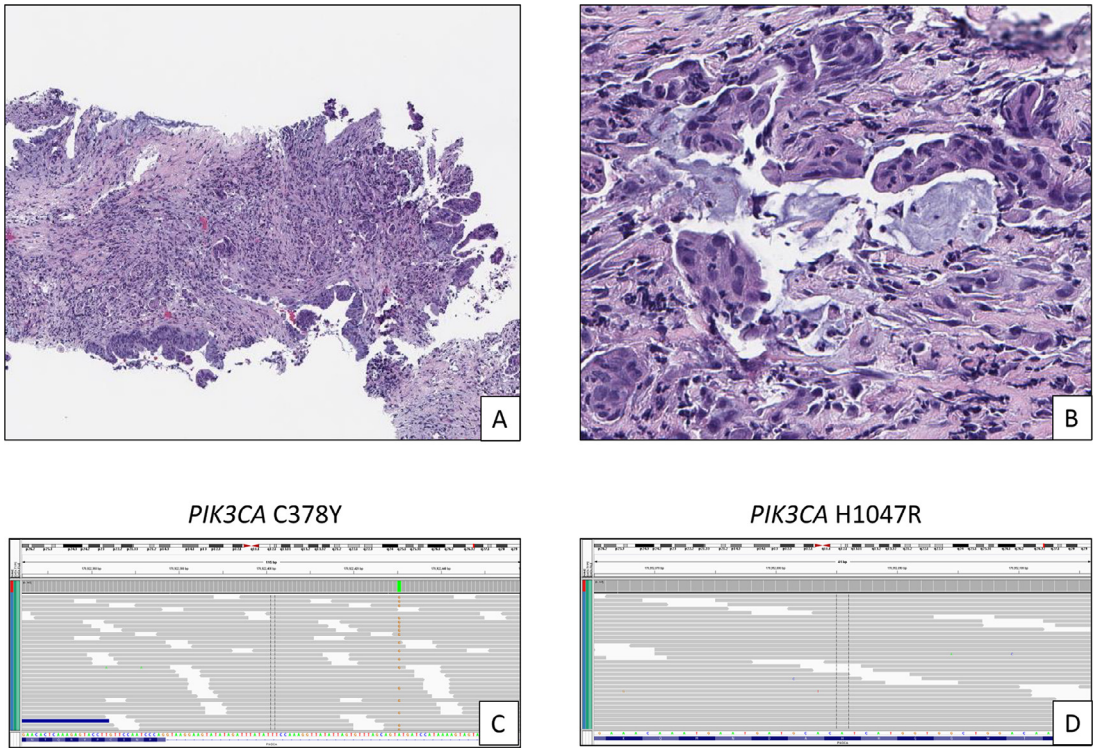

Fig. 5. Low (A) and high (B) magnification of a biopsy of a metastatic lesion in the pelvis of an 84-year-old man with a prior history of primary urethral adenocarcinoma is shown. This tumor was positive for CK7 and CK20 and negative for PSA and GATA-3 on IHC staining. Comprehensive genomic profiling revealed 2 distinct mutations in PIK3CA: a known to be activating C378Y base substitution (C) and a known to be activating H1047R base substitution (D) that has been associated with mTOR therapy response [23] as well as short variant mutations in SF3B1 (K700E) and SMAD2 (S464*). PIK3CA mutations have been detected in 15% of all urinary tract carcinoma including 5%−26% of bladder urothelial carcinomas and in 18% of renal pelvis urothelial carcinomas. Drugs targeting PIK3CA mutations have been approved for ER+ metastatic breast carcinoma. Recently, the frequency and positive impact on anti-PIK3CA-targeted therapy response of so-called "double-hit" PIK3CA mutations in metastatic breast carcinoma have been reported [22]. Double-hit PIK3CA mutations in urethral carcinomas have not been previously reported. It is possible that pan-cancer trials for double-hit PIK3CA mutations and new and existing anti-PIK3CA drugs will emerge in the near future.

subtypes feature contrasting genomic profiles, which have a significant impact on the selection of both targeted therapies and immunotherapies for these patients.

The UrthUC in both men and women feature genomic signatures that closely align with the genomic signatures of these tumor types widely reported for urinary bladder carcinomas [21,24−26]. These findings are also similar for the MSI status and TMB levels of these tumor types. The cause of the similarities of urinary bladder UC/SCC and urethral UC/SCC may likely be attributed to tobacco exposure in both patient groups. So-called "smoking genomic signatures" have been directly linked to UC and SCC of the urinary tract [27,28]. Similarly, tobacco exposure has been linked to the relatively higher median TMB levels in lung cancer (NSCLC) and tumors with TMB >20 mutations/Mb of sequenced DNA [18,29,30]. This study did not find a significant frequency of tobacco-associated gene signatures or differences in tobacco exposure among the 4 UrthCA subtypes. Although TP53 mutations are seen in all 4 types of UrthCA, mutations in the TERT gene were prominent only in UrthUC and UrthSCC and not in UrthAC and UrthCC. TERT mutations are well described in urinary bladder UC and SCC [21,24−26].

PIK3CA mutations were identified in all 4 UrthCa groups at frequencies ranging from a low in UrthAC (12%) to a high in UrthCC (31%). PIK3CA mutations have been detected in 15% of all urinary tract carcinoma including 5%−26% of bladder urothelial carcinomas and in 18% of renal pelvis urothelial carcinomas [21,24−26]. PIK3CA has long been considered a potential target for mTOR inhibitors with treatment results based on the presence of PIK3CA mutations varying in different tumor types [31−33]. More recently, the direct PIK3CA inhibitor alpelisib has been approved for the treatment of relapsed and metastatic ER+ breast cancer harboring PIK3CA mutations [34]. Novel anti-PIK3CA drugs are in late stages of clinical trials indicating that there may be a time in the future when a drug achieves a pan-cancer indication for tumors featuring PIK3CA mutations [35,36]. Fig. 5 describes a patient with clinically advanced UrthAC whose pelvic metastatic disease sequencing revealed 2 distinct mutations in PIK3CA (C378Y and H1047R) as well as short variant mutations in SF3B1 (K700E) and SMAD2 (S464*). Recent evidence has emerged that tumors that harbor 2 individual PIK3CA mutations tend to have less frequent additional driver mutations and appear to selectively identify these tumors for

robust and durable responses to PIK3CA inhibitors [22]. Finally, among other potential alterations that are documented as predictors of mTOR pathway inhibitors, mutations in TSC1 and TSC2 stand out in their association with benefit from treatments with drugs such as everolimus [37,38]. TSC1/2 are well documents in urinary bladder UC and were identified in from 3% to 8% of the UrthCa subtypes in this study. Fig. 2 describes a UrthCC patient whose tumor revealed a TSC2 mutation on CGP along with other potential genomic targets including PTCH1 and potentially VEGFA.

Both amplifications and short variant mutations in ERBB2 were identified in the UrthCa cases and were most frequent in the UrthAC cohort. In the UrthAC cases, the ERBB2 alterations were exclusively amplifications. Although responses to anti-HER2-targeted therapies with either antibody therapeutics and/or small molecule kinase inhibitors are described for bladder cancer patients, treatments of UrthCa patients with these drugs have not been widely described [39–41]. Fig. 4 describes a patient who presented with vulvovaginal Paget's disease and a deeply invasive URTHAC. CGP revealed 58 copies of the ERBB2 gene which strongly indicated this amplification as the main driver of this tumor and potential for responsiveness to anti-HER-targeting agents.

In recent years, tumors with BRCA1 and BRCA2 mutations, either somatic only or both germline and somatic, as featuring significant DNA damage repair capabilities and enhanced sensitivity to the PARP inhibitors class of anticancer drugs [20,42,43]. In Fig. 3, in addition to the high level of ERBB2 amplification, an inactivating point mutation in BRCA2 gene was identified. There are no published studies on the use of PARP inhibitors in UrthCa or large scale on strategies to combine anti-HER drugs with PARP inhibitors in other tumors that harbor both ERBB2 amplification and BRCA1 or BRCA2 mutations. However, the enhancement of trastuzumab efficacy with PARP inhibition in ERBB2 amplified/BRCA1/2 mutated preclinical models has been described [44].

The CGP assay used in this study identifies the MSI status, the TMB, and individual genes that have been linked to responsiveness and resistance to ICPI-based therapies. There were no MSI-high cases in the UrthCa patients. The TMB, in contrast, varied significantly with significantly higher TMB levels in the UrthUC and UrthSCC cases as described previously. The UrthAC and UrthCC cohorts featured no cases with TMB >20 mutations/Mb. The PD-L1 IHC staining using the Dako 22C3 staining kit and scoring limited to tumor cell membrane expression was available on a limited number of patients in each group and was significantly higher in the UrthSCC patients than in the other tumor types. Given publications in NSCLC, the UrthSCC cases with both high PD-L1 staining and TMB >20 mutations/Mb would be predicted to experience a robust and durable response to ICPUI treatments [19,45]. For individual genes associated with ICPI responses, STK11 and KEAP1 mutations associated with ICPI resistance were rarely identified and not >1% in frequency for any of the UrthCa subtypes. Although similarly uncommon, mutations in the PBRM1 gene linked to ICPI efficacy in clear cell renal cell carcinoma were identified in <1% of the UrthCa cases in this study. In Fig. 2, this UrthCC CGP featured an inactivation PBRM1 mutation which could be used as a possible ICPI efficacy signal for this patient whose tumor harbored multiple targeted therapy options.

Of all the UrthCa cases included in this study, the UrthCC subtype, at only 13 patients, included the smallest number of cases. UrthCC is a challenging pathologic diagnosis, especially in the differential consideration with UrthAC (NOS) [46–49]. Although significantly less common in men than women, UrthCC has been documented in men and distinguishing from prostatic adenocarcinoma invading the prostatic urthera [50–52]. In the current study, CGP of UrthCC revealed potentially targetable GA in the mTOR pathway (PIK3CA and TSC2) as well as potential kinase targetable GA in genes such as EGFR, PTCH1, and MET. The finding of PIK3CA/mTOR pathway mutations has been previously reported in UrthCC [53]. In addition, a previously published study of UrthCC using transcriptome analysis reported the presence of potential gene fusions for this tumor type, but no gene fusions were identified by the DNA sequencing only assay employed in the current study [23]. Finally, the absence of MSI high, high TMB levels, or significant PD-L1 expression in this small subset of patients indicates that clinically advanced UrthCC may not be responsive to immunotherapy regimens.

Limitations of the present study can be attributed to the referral case nature of our database. Consequently, data regarding urethral location of the primary tumor were largely unavailable. This was especially true for cases where a metastasis biopsy was provided rather than the original primary tumor specimen. Similarly, patient clinical background data was limited, e.g., history of radiation or recurrent urinary tract information.

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