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# Genomic Profiling of Chinese Urothelial Carcinoma Patients

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## Abstract

# Background

Urothelial carcinoma (UC) is the most common genitourinary malignancy in China. In this study, we studied the genomic features in Chinese UC patients, and investigated the concordance of genetic alterations between serum circulating tumor free DNA (ctDNA) and matched tumor tissue.

# Methods

A total of 112 UC patients were enrolled, and 31 of which were upper tract UC (UTUC) and 81 were UC of bladder (UCB), respectively. Utilizing target next-generation sequencing, we analyzed genomic alterations of 92 selected genes.

# Results

94.64%, 86.61% and 62.50% of patients in our cohort were identified as having valid, oncogenic or actionable somatic alterations, respectively, and the most altered genes were *TP53*, *KMT2D*, *KDM6A*, *FAT4*, *FAT1*, *CREBBP* and *ARID1A*. *HRAS* (22.0% vs 3.7%) and *KMT2D* (59.26% vs 34.57%) were more altered in UTUC than in UCB in our cohort. Comparisons of somatic alterations of UCB and UTUC between our cohort and western cohorts revealed significant differences in gene prevalence. Notably, 28.57%, 17.86% and 47.32% of cases harbored alterations in FGFRs, ERBBs and DNA damage repair genes, respectively. Furthermore, 75% of patients carried non-benign germline variants, but only two (1.79%) were pathogenic. By comparison of ctDNA and matched tumor tissue, the overall concordance for genomic alterations was 42.97% (0-100%). 47.25% of alterations detected in ctDNA were not detected in the matched tissue, and 25.58% of which were oncogenic.

# Conclusions

We found a unique genomic feature of Chinese UC patients. A good concordance of genomic features between ctDNA and tissue samples were identified.

## Background

Urothelial carcinoma (UC) is the most common genitourinary malignancy in the world [1]. As reported by the national cancer center of China, the estimated number of newly-diagnosed and mortality of urothelial carcinoma patients in 2015 was 80,500 and 32,900, respectively [2]. Most non-muscle invasive bladder cancer patients will have disease recurrence and progression to the invasive or metastatic disease within 5 years [3]. Treatments for locally advanced or metastatic disease have been dramatically changed in the

last three years, especially with the development and approval of the five immune checkpoint inhibitors (ICIs) and one FGFR inhibitor by the U.S. Food and Drug Administration (FDA).

Previous studies have widely characterized the genomic features of urothelial carcinoma, but the samples were mainly limited to the Caucasians [4]. To date, rare research had studied the molecular features of Chinese UC patients. The genomic alterations between western and Chinese UC patients may differ, especially considering the exposure of aristolochic acid and the different genetic backgrounds [5]. Meanwhile, as most of the genomic research in UC conducted on the tumor tissue samples, patients without sufficient and valid achieved tumor samples or unable to biopsy will miss the chance of target therapies. Recently, circulating cell-free tumor DNA (ctDNA) has been widely studied and applied in cancer diagnosis, treatment selection and monitoring, especially for lung cancer. For localized bladder cancer, ctDNA in plasma and urine was proved to be detectable even at the early phase of disease and associated with disease recurrence or progression [6]. However, whether ctDNA in blood or urine would be an effective substitution for tissue for genetic testing in UC is still unknown.

In the present study, we analyzed the germline and somatic gene alterations in 112 Chinese UC patients. The basic genomic alteration profiles were described and compared with corresponding Western data. Meanwhile, we compared the concordance of genetic alterations between ctDNA in plasma and matched tumor tissue to comprehend the utility of liquid biopsy samples in UC patients.

## Methods

# Samples source and ethic data

In total, formalin-fixed, paraffin-embedded (FFPE) tumor tissues and matched blood in EDTA (for germline tests) from 112 diagnosed UC patients (Supplemental Table 1) were collected with the signing of informed consent and agreements to publish the results for research purpose. All tumor tissues were pathologically reviewed to contain at least 20% tumor cells. 34 patients who cannot provide sufficient or valid tumor tissue samples were collected blood for ctDNA testing instead. Among enrolled patients, 20 of them agreed to provide additional blood samples for comparison of the genomic differences between blood and matched tumor.

# Dna Isolation

The FFPE tissues and peripheral blood mononuclear cells were collected to extract DNA using DNeasy Blood & Tissue Kit (Qiagen, Inc.) under the manufacturer's instructions. Cell-free DNA (cfDNA) was extracted from serum using the QIAamp Circulating Nucleic Acid Kit (Qiagen, Inc.) following the protocol of the manufacturer. The purified gDNA and cfDNA were quantified using the Qubit 3.0 Fluorometer (Life Technologies, Inc.) and StepOnePlus System (Life Technologies, Inc.).

# Target Next-generation Sequencing

For the matched germline and tumor samples, 100 ng of DNA was sheared with a Covaris E210 system (Covaris, Inc.) to get 200 bp fragments. We performed next generation sequencing of tumor and gDNA matched germline DNA using Accel-NGS 2S DNA Library Kit (Swift Biosciences, Inc.) for library preparation and xGen Lockdown Probes kit (IDT, Inc.). The custom xGen Lockdown probe was synthesized by IDT, Inc. for the exons and selected intronic regions of 92 genes (Supplemental Table 2). The prepared library was quantified using the Qubit 3.0 Fluorometer (Life Technologies, Inc.), and quality and fragment size were measured using Agilent 2100 Bioanalyzer (Agilent Technologies, Inc.). Samples underwent paired-end sequencing on an Illumina Nextseq CN500 platform (Illumina Inc) with a 150-bp read length. Mean coverage of 1260.5×, 3759.3 × and 223.6 × were achieved for tumor gDNA, blood cfDNA and peripheral blood mononuclear cells gDNA, respectively.

# Data Processing

Raw sequencing data were aligned to the reference human genome (UCSC hg19) by Burrows-Wheeler Aligner. After removing duplicate and local realignment, we applied Genome Analysis Toolkit (GATK) for single nucleotide variation (SNV), insertion and deletion (inDel) calling. Next, after removing the germline alterations from matched blood samples, the somatic alterations were obtained. Variants were annotated using the ANNOVAR software tool. Copy number was analyzed using CNVkit. Genomic alterations data from The Cancer Genome Atlas database (TCGA) and Memorial-Sloan Kettering Cancer Center (MSKCC) was downloaded from cBioPortal (http://www.cbioportal.org).

# Statistical analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) statistical package and Graphpad (Prizm 8). Differences were considered significant if $p < 0.05$.

## Results

# Demographic and clinical data

The demographic and clinical data of patients enrolled in our cohort were summarized in Table 1. Of the enrolled patients, 72.32% were bladder cancer (81/112), and the rest were diagnosed as upper tract UC (27.68%, 31/112), including carcinoma of the renal pelvis and ureteral. The median age at diagnosed was 67 (range 25–86). The sex ratio between males and females was 2.61:1, which was close to the sex ratio (2.97:1) in Chinese UC patients reported by the national cancer center of China. The amounts of enrolled smoker and non-smoker were the same (56 versus 56).

# Somatic Alterations In Chinese Uc Patients

In total, 106 of 112 samples (94.64%) had been identified with valid somatic alterations. The mean and median counts of somatic alterations per sample were 6.05 and 4, respectively. Six samples were negative for somatic mutations identification after excluding the germline alterations. The most frequently mutated genes in our cohort were *TP53* (48%), *KMT2D* (43%), *KDM6A* (23%), *FAT4* (21%), *CREBBP* (20%), *ARID1A* (19%) and *FAT1* (18%), respectively (Fig. 1A). Notably, 62.50% (70/112) and 86.61% (97/112) patients in our cohort were identified as having actionable and oncogenic alterations according to the OncoKB database, respectively [7]. No significant difference was found in gene prevalence between low- and high-grade urothelial carcinoma. Pathway analysis indicated that the most frequently enriched pathways were Chromatin regulatory (73.21%), RTK-RAS-MAPK (62.50%), cell cycle (53.57%), DNA damage repair (DDR, 47.32%), PI3K-mTOR (33.93%) and Wnt (32.14%), respectively (Fig. 1B). We didn't find any significant genomic difference between high- and low-grade UC patients.

# Germline Alteration

Excluded of variants identified as benign or likely benign according to the American College of Medical Genetics and Genomics (ACMG) guideline, 75.89% (85/112) patients in our cohort harbored at least one germline alteration (Supplemental Table 3). After exclusion of known and likely benign variants, 244 germline variants were identified. However, only two patients (1.85%) had variants that could be identified as pathogenic or likely pathogenic, including a *ERCC4*-p. Lys481fs and a *BRCA2*- p.Thr3030fs (Supplemental Table 3). The patient harbored deleterious *BRCA2* germline variants was also concurrent with prostate cancer. None pathogenic or likely pathogenic germline variant associated with Lynch syndrome, including *MLH1*, *MSH2*, *MSH6* and *PMS2* genes, was identified in UTUC and UC patients of our cohort.

**Differences of somatic gene alterations in UC patients between our cohort and Western cohort**

To determine the potential difference of genomic features between Western and Chinese UC patients, we compared the alterations data of the selected 92 genes between our cohort and Western cohorts (UC data published by TCGA and UTUC data published by MSKCC). The prevalence of alterations in *FGFR4*, *KDM5C*, *TERT*, *PDGFRB*, *FLT3*, *FLCN*, *MSH6*, *FLT1* were higher in UCB patients in our cohort, compared with TCGA (Fig. 2A). As for UTUC, a higher mutated frequency of multiple genes, including *TP53*, *LRP1B*, *KMT2D*, *FAT4*, *BRCA1*, *FGFR2* and *BRIPI*, were found in our cohort (Fig. 2B). On the contrary, the mutation frequency of *FGFR3* gene in the MSKCC cohort was three times higher than that in our cohort (48.24% versus 16.13%, $p < 0.001$).

# Differences Of Somatic Gene Alterations Between Utuc And Ucb

To elucidate the differences of somatic gene alterations between UTUC and UCB, we compared the alterations from 81 UCB samples and 31 UTUC samples in our cohort. The genomic features of UCB and

UTUC were similar, while *KMT2D* (34.57% vs 64.52%, p < 0.01), *HRAS* (3.7% vs 19.35%, p < 0.01) and *CDKN2A* (2.47% vs 12.9%, p < 0.05) were more mutated in UTUC than in UCB (Fig. 2C). A higher mutated frequency of *HRAS* was also presented in UTUC compared with UCB in Western cohorts, but with additionally significantly different prevalence in *FGFR3*, *TP53*, *TERT*, *RB1*, *LRP1B*, *FAT4, KMT2A*, *ARID1A*, *BRIP1*, *TSC1* genes (Fig. 2D).

# Genomic Alterations In Fgfr Genes

28.57% (32/112) patients had at least one somatic alteration in FGFR genes (including *FGFR1*, *FGFR2*, *FGFR3* and *FGFR4*), but only 11 patients' alternations could be defined as gain of function (Fig. 3A). Notably, six of these patients had multiple oncogenic alterations in FGFR genes. Same to previous studies' results, the most altered gene was *FGFR3* (13.39%). Hotspot variants, including *FGFR3*-p.Ser249Cys, p.Arg248Cys and p.Tyr373Cys were identified in six samples, while *FGFR3*-TACC3 fusion was only identified in two patients. Three novel variants (defined as unreported in any other research or single nucleotide polymorphism database before and without ExAC frequency), including p.His349Asn, p.Val166Met and p.Thr755Lys of *FGFR3* were identified, though the functions were still unknown (Fig. 3B). Nine patients (8.04%) were identified with *FGFR2* alterations, of which only three alterations could be defined oncogenic, including one p.Asn549Lys, one p.Lys659Met and one copy number gain (copy number 32.87). No *FGFR2* fusion was identified. Four novel variants with unknown function, including p.Gly305Arg, p.Tyr207Phe, p.Met803Leu and p.Gln683Ter of *FGFR2* gene were identified in this study. Only six patients carried *FGFR1* gene alterations, and half of them were amplification. Eight patients (all were bladder cancer) carried nonsynonymous single nucleotide variants with unknown function in *FGFR4* gene.

# Genomic Alterations In Erbb Family

20 of our cases (17.86%) carried somatic alterations in the ERBB genes (*EGFR, ERBB2* and *ERBB3*), and three of them had dual alterations (Fig. 3C). Five patients (4.46%) carried *EGFR* gene alteration and two of them had EGFR copy number gain. Notably, one patient had an activated *EGFR* exon 20 insertion (*EGFR*-p.Val769_Asp770insAspAsnPro) and copy number gain. We observed *ERBB2* alterations in 12 patients (10.71%) s, and most of the oncogenic alterations located in furin-like cysteine rich region (amino acid 190–343) and protein tyrosine kinase domain (amino acid 721–975) (Fig. 3D). Two of the five identified *ERBB3* alterations were in the furin-like cysteine rich region (amino acid 182–332) and oncogenic.

# Somatic Alterations In Dna Repair Pathway

We observed 47.32% of cases had alterations in the DDR pathway as defined by the MSKCC DDR gene panel [8], including 37 (45.68%, 37/81) UCB patients and 16 (51.61%,16/31) UTUC patients, respectively.

Distribution of alterations among specific DDR pathway was similar, with slight enrichment in Fanconi anemia (FA) pathway (Fig. 4A). The most frequently mutated DDR genes were *BRCA2* (10.71%), *ATM* (9.82%), *ERCC2* (8.93%), *BRCA1* (7.14%) and *BRIP1* (6.25%), respectively (Fig. 4B). Among patients carried DDR gene alterations, only 16 of them (14.29%) had at least one known or likely deleterious somatic DDR alterations (Fig. 4C). The distribution of specific genes was exhibited in Fig. 5C, and the most frequently mutated DDR genes with known or likely deleterious variants were *ATM* (n = 7, 31.82%) and *BRCA2* (n = 5, 22.73%). Eighteen patients (16.07%) carried somatic alterations in DNA mismatch repair (MMR) genes, including in *MLH1*, *MSH2*, *MSH6* and *PMS2*. The majority of these MMR gene alterations were unknown of function, with four exceptions of alterations in *MSH2* and *MLH1*, which could be defined as loss of function.

### Concordance of genetic alterations between ctDNA in serum and matched tumor tissue

Twenty matched tumor tissue and blood samples were collected to comprehend the concordance and discordance of genomic alterations between different sample types in UC. Only three patients' matched samples were collected with an interval time over one month (7 months for P074, 18 months for P084 and 34 months for P110, respectively). In total, 99 and 91 somatic alterations were identified in tumor and serum ctDNA samples from 20 patients who underwent genomic testing for matched tumor tissue and serum ctDNA samples, respectively. Only one patient's ctDNA was negative for valid somatic alterations identification, while matched tumor samples were positive for alterations calling. Conversely, in Patient 20, ctDNA analysis revealed oncogenic mutations in *PIK3CA*, *TP53*, *ARID1A* and *KDM6A* with allele fractions all beyond 3%, for which there was no corresponding valid alteration in the matched tissue sample (Fig. 5A). There was no significant difference between tissue and blood in the median number of genomic alterations (4 versus 4). By comparison of blood and matched tumor tissue, the overall concordance for genomic alterations and altered genes identified in matched samples was 42.97% (0-100%) and 46.83% (0-100%), respectively (Table 2). Among the genomic alterations, 48 of them were in concordance between ctDNA and tumor tissue. Fifteen actionable alterations were identified in tissue defined according to the Oncobkb database, and 60% of them were shared in matched ctDNA (Fig. 5B). Notably, 41 of 91 (48.35%) alterations detected in ctDNA were not detected in the corresponding tissue sample. Specifically, 12 of them (27.27%) were oncogenic mutations and 3 of 44 (6.82%) could be identified as actionable, including a *PIK3CA* activated hot-spot, *PTEN* frameshift and *KDM6A* frameshift mutation.

## Discussion

Compared with UC patients in Western countries, more Chinese UC patients were diagnosed in the advanced stage for multiple reasons [9]. Meanwhile, fewer drugs were developed and approved in China for advanced UC which left no choice but only chemotherapy for most advanced Chinese UC patients. Meanwhile, because of the aristolochic acid exposure history, many Chinese UC patients, especially UTUC, tend to have chronic kidney disease, which made them unfit for platinum treatment [5]. Therefore, it's of great importance to understand the genomic features of Chinese UC patients for further therapy

development and clinical trials. Remarkably, over 60% of UC patients in this study were identified to have at least one potentially actionable somatic alteration, which would afford more choice for further treatment.

To date, *FGFR2* and *FGFR3* genes were the most actionable genes in UC, especially for UTUC, as erdafitinib (a *FGFR* inhibitor) was proved to have a 40% objective response rate in previously treated locally advanced and unresectable or metastatic UC with *FGFR*2/3 alterations [10]. We identified *FGFRs* gene alteration in 26.85% of UC patients, which was close to the corresponding ratio in the TCGA database [4]. Though may have an increased sensitivity to FGFR inhibitor, UC patients with *FGFRs* alterations may have a lower response rate to the ICIs therapy because of the suppressed infiltration of tumor immune cells in the tumor environment [11, 12]. Meanwhile, alterations in ERBB pathway, especially in *ERBB2*, raised increasing interest in UC recently, as 12.4% and 11% UCB patients had ERBB2 overexpressing (as tested by HER2 immunohistochemistry (IHC) staining) and mutations, respectively [13]. Similarly, ERBB2 alteration was observed in 10.71% of cases, which was equal to the data in Western UC patients[4]. An interesting correlation between *ERBB2* gene mutations and a higher response possibility to platinum-based neoadjuvant chemotherapy was found in 61 muscle-invasive UCB [14]. Meanwhile, anti-Her2 therapy by Trastuzumab combined with chemotherapy has been proved to have an outstanding response rate and median overall survival of 70% and 14.1 months, respectively, in 44 advanced UC patients with HER2 overexpression in a single arm phase II trial [15]. Other anti-Her2 therapies, such as tyrosine kinase inhibitors, antibodies and antibody-drug conjugate, had been widely developed in UC with abnormal *ERBB2* [16].

As previous work reported, DDR alterations were associated with a higher response rate and clinical benefit for both immune checkpoint inhibitors (ICIs), platinum-based neoadjuvant therapy and first-line chemotherapy in UC patients [17]. The previous study found 23.9% UCB patients had alterations in Homologous recombination repair gene pathway [18]. In our study, we found 47.32% and 14.29% UC patients had alterations and deleterious alterations in DDR pathway, respectively. The ratios were similar to the results analyzed by the panel involved the same genes [19]. Except for ICIs, tumors with deleterious DDR gene mutations, especially for BRCA1/2 gene, were also associated with better sensitivities with poly (ADP ribose) polymerase inhibitors (PARPi) [20]. Though it is still lack of evidence that proved the efficiency of PARPi in UC patients, PARPi combined with ICIs has been demonstrated to have cooperative effects in treating UC patients with HR mutations [21].

The prevalence of germline mutations of cancer susceptibility genes in patients with unselected UC was conflicting. Although previous studies established the point that UC was a carcinoma with rarely cancer susceptibility genes alterations, the latest research found that 14% of 586 unselected UC patients carried pathogenic or likely pathogenic germline variants, and 11.26% (66/586) of them had deleterious variants in DNA repair pathway [22]. Until now, the prevalence of germline mutations of cancer susceptibility genes in Chinese UC patients remains unclear. In our cohort, we only identified 1.79% of patients with pathogenic or likely pathogenic germline variants, which may represent a low prevalence of cancer susceptibility genes in Chinese UC patients compared to the corresponding Caucasian patients. In this

study, we found a novel likely-pathogenic *ERCC4* gene germline variants in a UTUC patient. Previously, *ERCC4* gene mutations were identified in fanconi anemia, skin-photosensitive, nucleotide excision repair (NER)-deficient disorders xeroderma pigmentosum and XFE progeroid syndrome [23]. 2.03% of UC patients had alterations in ERCC4 gene, while no deleterious germline ERCC4 carrier of UC had been identified [24]. Meanwhile, Previous research found that 7%-8.3% unselected UTUC patients had lynch syndrome-related features, such as deficient mismatch repair (dMMR), microsatellite instability high (MSI-H) or deleterious alterations of MMR genes, while only 2.1% occurred in UC patients [22, 25]. In the present research, no germline variant in mismatch repair pathway was found, and the possible reasons may lie in the differences between Chinese and Western UC patients, limited UTUC patients enrolled and technology limitations (such as lack of detecting in *MLH1* promoter hypermethylation and large rearrangements) [26].

Moreover, though previous studies suggested that ctDNA could serve as an effective biomarker in cancer diagnosis, risk stratification, therapy monitoring and early relapse in UC, the controversy about liquid biopsy remained [27]. Several prior studies published controversial results about the concordance levels between tissue and blood in other genitourinary carcinomas [28]. We found a concordance of 42.97% (0-100%) of the genetic alterations between 20 ctDNA and matched tissue samples, which was not reported before. Though factors, including tumor stage, metastasis status treatments, the interval time of sample collection and analyzed panel of genes would affect the concordance of genomic alterations between ctDNA and tissue, lipid biopsy is still an expanding way to overcome the intratumoral and intertumoral heterogeneity {Meeks, 2020 #632}. Our result support that genetic testing on tissue is still the gold standard for UC patients, meanwhile liquid biopsy would serve as a complementary source to provide additional and meaningful results to comprehend the genomic feature more precisely.

# Conclusion

In this study, we identified a unique genomic feature of germline and somatic alterations in Chinese UC patients by next-generation sequencing. In total, 62.50% of 112 Chinese UC patients had at least one actionable genomic alteration, which would benefit from matched or related target therapies. Furthermore, our study found a good genomic concordance between ctDNA and matched tumor, suggesting the potential application of liquid biopsy in UC.

# Abbreviations

**UC** urothelial carcinoma

**ICI** immune checkpoint inhibitor

**FGFR** fibroblast growth factor receptor

**FDA** Food and Drug Administration

**ctDNA** cell-free tumor DNA

**FFPE** paraffin-embedded

**EDTA** ethylenediaminetetraacetic acid

**cfDNA** Cell-free DNA

**GATK** Genome Analysis Toolkit

**SNV** single nucleotide variation

**inDel** insertion and deletion

**TCGA** The Cancer Genome Atlas database

**MSKCC** Memorial-Sloan Kettering Cancer Center

**SPSS** Statistical Package for the Social Sciences

**DDR** DNA damage repair

**ACMG** American College of Medical Genetics and Genomics

**UTUC** upper tract urothelial carcinoma

**UCB** urothelial carcinoma of bladder

**MMR** mismatch repair

**dMMR** deficient mismatch repair

**NER** nucleotide excision repair

**MSI-H** microsatellite instability high

# Declarations

### Ethics approval and consent to participate

Allthe subjects have signed informed consent for inclusion before participating in the study. The study was performed in accordance with the Declaration of Helsinki and was approved by the Medical Ethics Committee of PLA General Hospital for Clinical Investigation.

### Consent for publication

Not applicable.

### Availability of data and material

All data generated or analysed during this study are included in this published article and its supplementary information files.

### Competing Interest

The authors indicated no financial relationship.


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### Author Contributions

BY, XZ and CW contributed equally to this work.


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Not applicable


# References


1. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.
2. Chen W, Zheng R, Baade PD, Zhang S, Zeng H, Bray F, Jemal A, Yu XQ, He J. Cancer statistics in China, 2015. CA Cancer J Clin. 2016;66(2):115–32.
3. Babjuk M, Burger M, Comperat EM, Gontero P, Mostafid AH, Palou J, van Rhijn BWG, Roupret M, Shariat SF, Sylvester R, et al. European Association of Urology Guidelines on Non-muscle-invasive Bladder Cancer (TaT1 and Carcinoma In Situ) – 2019 Update. Eur Urol. 2019;76(5):639–57.
4. Network TCGAR. Comprehensive molecular characterization of urothelial bladder carcinoma. Nature. 2014;507(7492):315–22.
5. Xiong G, Chen X, Li X, Fang D, Zhang L, Yang L, Zhang L, Yao L, He Z, Zhou L. Prevalence and factors associated with baseline chronic kidney disease in China: a 10-year study of 785 upper urinary tract urothelial carcinoma patients. J Formos Med Assoc. 2014;113(8):521–6.
6. Birkenkamp-Demtroder K, Christensen E, Nordentoft I, Knudsen M, Taber A, Hoyer S, Lamy P, Agerbaek M, Jensen JB, Dyrskjot L. Monitoring Treatment Response and Metastatic Relapse in Advanced Bladder Cancer by Liquid Biopsy Analysis. Eur Urol. 2018;73(4):535–40.
7. Chakravarty D, Gao J, Phillips S, Kundra R, Zhang H, Wang J, Rudolph JE, Yaeger R, Soumerai T, Nissan MH, et al: **OncoKB: A Precision Oncology Knowledge Base**. *JCO Precision Oncology*

2017(1):1–16.

8. Teo MY, Seier K, Ostrovnaya I, Regazzi AM, Kania BE, Moran MM, Cipolla CK, Bluth MJ, Chaim J, Al-Ahmadie H, et al. Alterations in DNA Damage Response and Repair Genes as Potential Marker of Clinical Benefit From PD-1/PD-L1 Blockade in Advanced Urothelial Cancers. J Clin Oncol. 2018;36(17):1685-+.
9. Pang C, Guan Y, Li H, Chen W, Zhu G. Urologic cancer in China. Jpn J Clin Oncol. 2016;46(6):497–501.
10. Loriot Y, Necchi A, Park SH, Garcia-Donas J, Huddart R, Burgess E, Fleming M, Rezazadeh A, Mellado B, Varlamov S, et al. Erdafitinib in Locally Advanced or Metastatic Urothelial Carcinoma. N Engl J Med. 2019;381(4):338–48.
11. Kim YW, Rose TL, Eckstein FR, Jarczyk M, Zengerling J, Sikic F, Breyer D, Bolenz J, Hartmann C A et al: **Predictive value of fibroblast growth factor receptor (FGFR) alterations on anti-PD-(L)1 treatment outcomes in patients (Pts) with advanced urothelial cancer (UC): Pooled analysis of real-world data**. *Journal of Clinical Oncology* 2020, **38**(suppl 6):abstr 493.
12. Robinson BD, Vlachostergios PJ, Bhinder B, Liu W, Li K, Moss TJ, Bareja R, Park K, Tavassoli P, Cyrta J, et al. Upper tract urothelial carcinoma has a luminal-papillary T-cell depleted contexture and activated FGFR3 signaling. Nature communications. 2019;10(1):2977.
13. Yan M, Schwaederle M, Arguello D, Millis SZ, Gatalica Z, Kurzrock R. HER2 expression status in diverse cancers: review of results from 37,992 patients. Cancer Metastasis Rev. 2015;34(1):157–64.
14. Groenendijk FH, de Jong J, Fransen van de Putte EE, Michaut M, Schlicker A, Peters D, Velds A, Nieuwland M, van den Heuvel MM, Kerkhoven RM, et al. ERBB2 Mutations Characterize a Subgroup of Muscle-invasive Bladder Cancers with Excellent Response to Neoadjuvant Chemotherapy. Eur Urol. 2016;69(3):384–8.
15. Hussain MH, MacVicar GR, Petrylak DP, Dunn RL, Vaishampayan U, Lara PN Jr, Chatta GS, Nanus DM, Glode LM, Trump DL, et al. Trastuzumab, paclitaxel, carboplatin, and gemcitabine in advanced human epidermal growth factor receptor-2/neu-positive urothelial carcinoma: results of a multicenter phase II National Cancer Institute trial. J Clin Oncol. 2007;25(16):2218–24.
16. Oh DY, Bang YJ. HER2-targeted therapies - a role beyond breast cancer. Nat Rev Clin Oncol. 2020;17(1):33–48.
17. Plimack ER, Dunbrack RL, Brennan TA, Andrake MD, Zhou Y, Serebriiskii IG, Slifker M, Alpaugh K, Dulaimi E, Palma N, et al. Defects in DNA Repair Genes Predict Response to Neoadjuvant Cisplatin-based Chemotherapy in Muscle-invasive Bladder Cancer. Eur Urol. 2015;68(6):959–67.
18. Heeke AL, Pishvaian MJ, Lynce F, Xiu J, Brody JR, Chen WJ, Baker TM, Marshall JL, Isaacs C: **Prevalence of Homologous Recombination-Related Gene Mutations Across Multiple Cancer Types**. *JCO Precis Oncol* 2018, **2018**.
19. Teo MY, Seier K, Ostrovnaya I, Regazzi AM, Kania BE, Moran MM, Cipolla CK, Bluth MJ, Chaim J, Al-Ahmadie H, et al. Alterations in DNA Damage Response and Repair Genes as Potential Marker of

Clinical Benefit From PD-1/PD-L1 Blockade in Advanced Urothelial Cancers. J Clin Oncol. 2018;36(17):1685–94.

20. Mateo J, Porta N, Bianchini D, McGovern U, Elliott T, Jones R, Syndikus I, Ralph C, Jain S, Varughese M, et al. Olaparib in patients with metastatic castration-resistant prostate cancer with DNA repair gene aberrations (TOPARP-B): a multicentre, open-label, randomised, phase 2 trial. Lancet Oncol. 2020;21(1):162–74.
21. Powles TB, Balar A, Gravis G, Jones R, Ravaud A, Florence J, Grivas P, Petrylak DP, Galsky M, Carles J, et al. An adaptive, biomarker directed platform study in metastatic urothelial cancer (biscay) with durvalumab in combination with targeted therapies. Ann Oncol. 2019;30(suppl_5):v356–402.
22. Carlo MI, Ravichandran V, Srinavasan P, Bandlamudi C, Kemel Y, Ceyhan-Birsoy O, Mukherjee S, Mandelker D, Chaim J, Knezevic A, et al. Cancer Susceptibility Mutations in Patients With Urothelial Malignancies. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 2020;38(5):406–14.
23. Bogliolo M, Schuster B, Stoepker C, Derkunt B, Su Y, Raams A, Trujillo JP, Minguillon J, Ramirez MJ, Pujol R, et al. Mutations in ERCC4, encoding the DNA-repair endonuclease XPF, cause Fanconi anemia. Am J Hum Genet. 2013;92(5):800–6.
24. Consortium APG. AACR Project GENIE: Powering Precision Medicine through an International Consortium. Cancer Discov. 2017;7(8):818–31.
25. Harper HL, McKenney JK, Heald B, Stephenson A, Campbell SC, Plesec T, Magi-Galluzzi C. Upper tract urothelial carcinomas: frequency of association with mismatch repair protein loss and lynch syndrome. Mod Pathol. 2017;30(1):146–56.
26. Mork M, Hubosky SG, Roupret M, Margulis V, Raman J, Lotan Y, O'Brien T, You N, Shariat SF, Matin SF. Lynch Syndrome: A Primer for Urologists and Panel Recommendations. J Urol. 2015;194(1):21–9.
27. Christensen E, Birkenkamp-Demtroder K, Sethi H, Shchegrova S, Salari R, Nordentoft I, Wu HT, Knudsen M, Lamy P, Lindskrog SV, et al. Early Detection of Metastatic Relapse and Monitoring of Therapeutic Efficacy by Ultra-Deep Sequencing of Plasma Cell-Free DNA in Patients With Urothelial Bladder Carcinoma. J Clin Oncol. 2019;37(18):1547–57.
28. Maia MC, Salgia M, Pal SK. **Harnessing cell-free DNA: plasma circulating tumour DNA for liquid biopsy in genitourinary cancers**. *Nature Reviews Urology* 2020.

# Tables

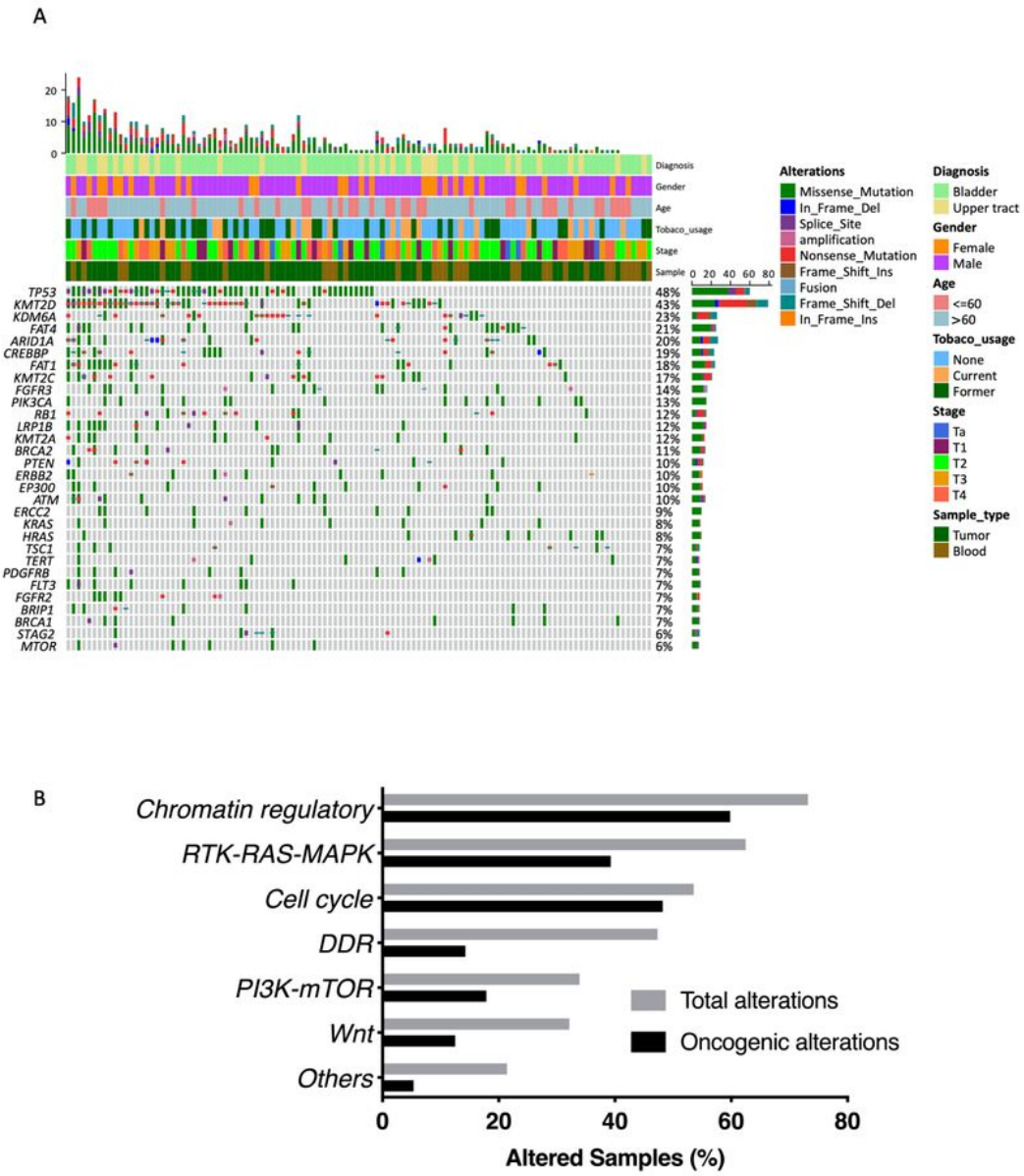
| Table 1 Demographic and clinical data of patients | | |
|---|---|---|
| Characteristics | | n=112 |
| Median age (range) | | 67 (25-86) |
| Sex | | |
| | Male | 81 |
| | Female | 31 |
| Smoking | | |
| | Current | 19 |
| | Former | 37 |
| | None | 56 |
| Tumor Stage | | |
| | T1 | 17 |
| | T2 | 43 |
| | T3 | 27 |
| | T4 | 19 |
| | Ta | 6 |
| Classification | | |
| | Upper tract | 31 |
| | Bladder | 81 |
| Sample Type | Blood | 34 |
| | Tumor | 78 |
| | | |

Table 2. Genomic alterations identified in matched tissue and blood

| Patient number | Genomic alterations in tissue number | Genomic alterations in blood number | Genes altered in both samples, number (%) | Genomic alterations in both samples, number (%) |
|---|---|---|---|---|
| P003 | 6 | 5 | 5 (83.3%) | 5 (83.3%) |
| P009 | 2 | 7 | 2 (28.57%) | 2 (28.57%) |
| P020 | 0 | 10 | 0 | 0 |
| P024 | 2 | 2 | 0 | 0 |
| P035 | 1 | 1 | 1 (100%) | 1 (100%) |
| P043 | 4 | 3 | 0 | 0 |
| P053 | 5 | 9 | 5 (62.5%) | 3 (27.27%) |
| P054 | 4 | 4 | 4 (100%) | 4 (100%) |
| P071 | 3 | 4 | 2 (50%) | 2 (40%) |
| P072 | 3 | 3 | 3 (100%) | 3 (100%) |
| P073 | 3 | 3 | 3 (100%) | 3 (100%) |
| P074 | 15 | 10 | 4 (36.36%) | 6 (30%) |
| P077 | 5 | 5 | 4 (66.67%) | 4 (66.67%) |
| P084 | 17 | 4 | 2 (13.33%) | 2 (9.52%) |
| P095 | 4 | 7 | 4 (57.14%) | 4 (57.14%) |
| P096 | 4 | 1 | 0 | 0 |
| P109 | 9 | 11 | 7 (63.64%) | 7 (63.64%) |
| P110 | 3 | 1 | 1 (50%) | 1 (33.33%) |
| P111 | 4 | 0 | 0 | 0 |
| P112 | 5 | 1 | 1 (25%) | 1 (20%) |

# Figures

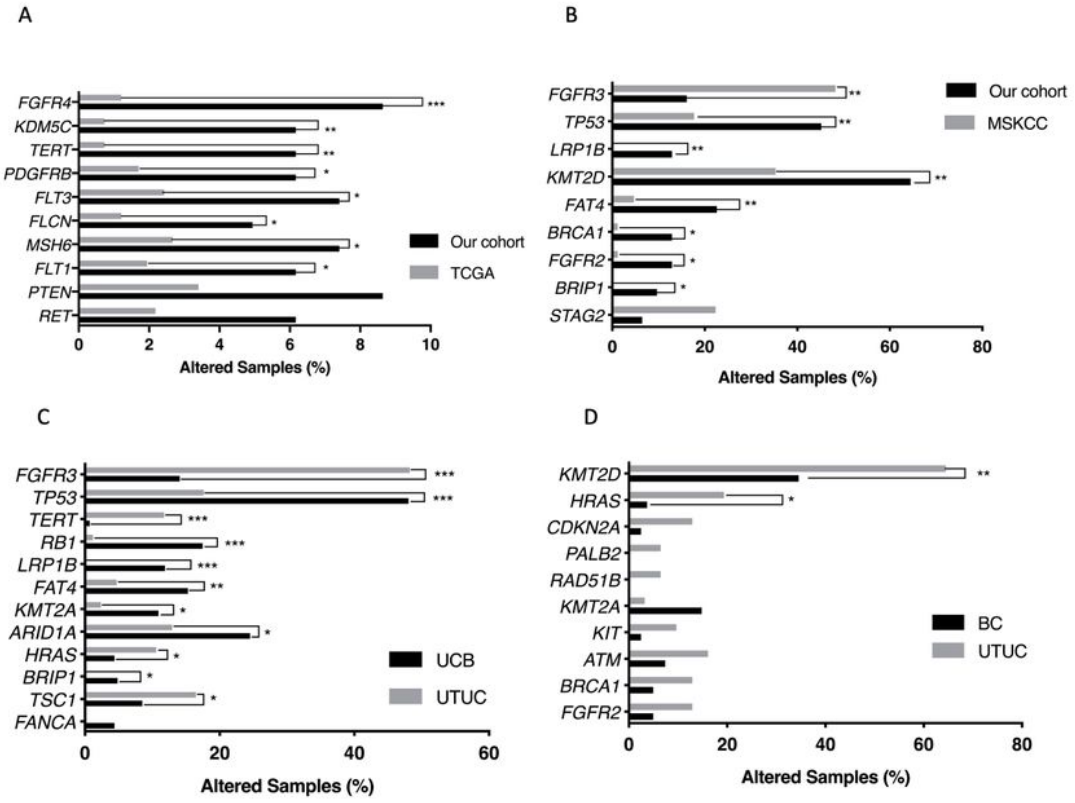
Figure 1




## Figure 1

(A) Oncoprint representation of the 92 genes in 112 UC patients. Alterations were categorized as missense mutations (green), in frame deletion (dark blue) or insertion (orange), frameshift deletion (grey blue) or insertion (brown) and nonsense mutation (red). (B) The distribution of genomic alterations in the identified pathways.

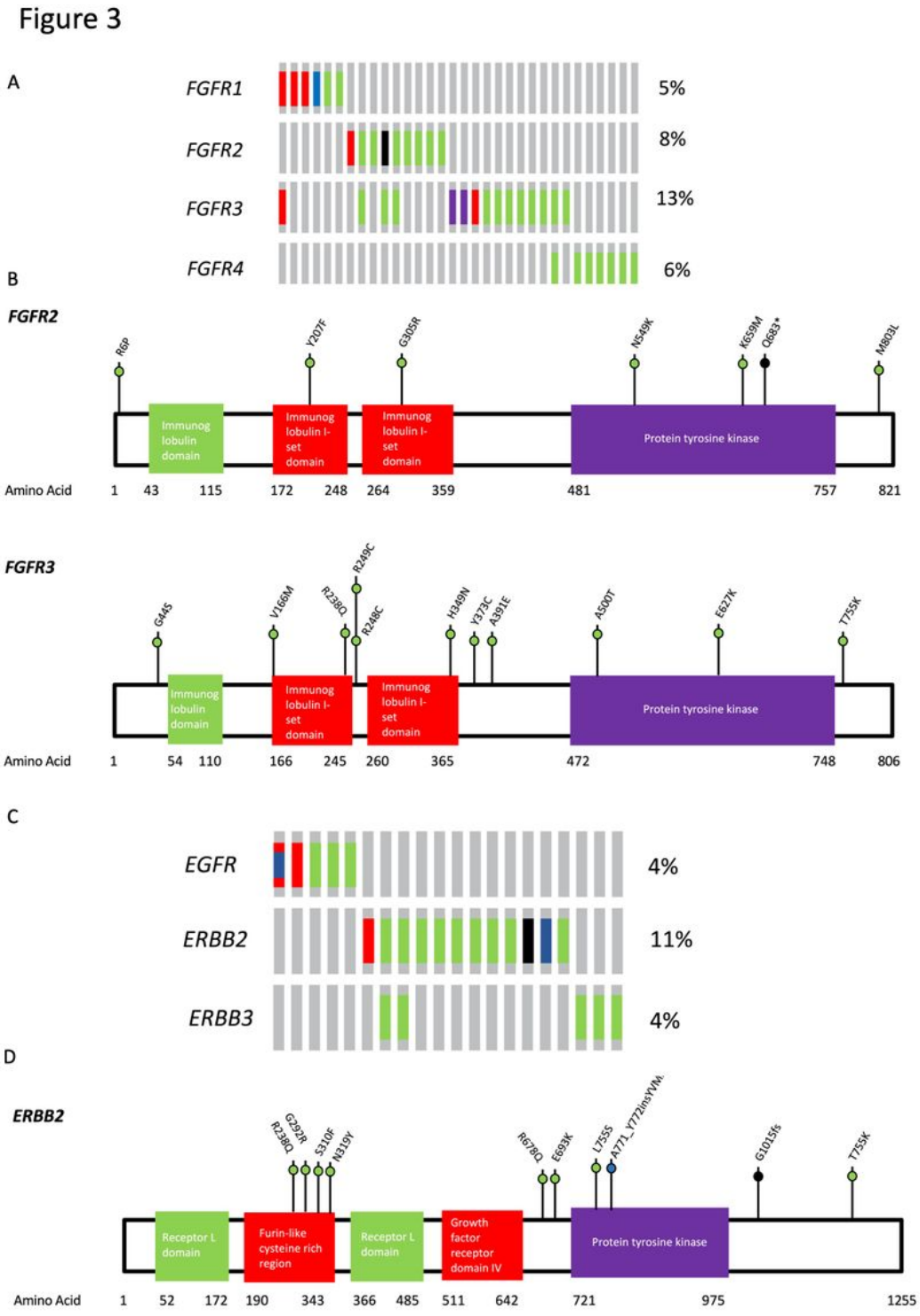
Figure 2




## Figure 2

(A) Comparisons of the gene prevalence identified in UCB between our cohort (black bars) and TCGA database (grey bars). (B) Comparisons of the gene prevalence identified in the UTUC between our cohort (black bars) and MSKCC cohort (grey bars). Two-sided Fisher's tests were conducted to compare the different frequency between two cohorts. UCB: urothelial carcinoma of bladder; UTUC: upper tract urothelial carcinoma. (C) Comparisons of the gene prevalence identified between the UCB and UTUC in

our cohort (D) and in western cohort(B). Two-sided Fisher's tests were conducted to compare the different frequency between two cohorts. UCB: urothelial carcinoma of bladder; UTUC: upper tract urothelial carcinoma. *** p≤0.001, ** p≤0.01, * p≤0.05.

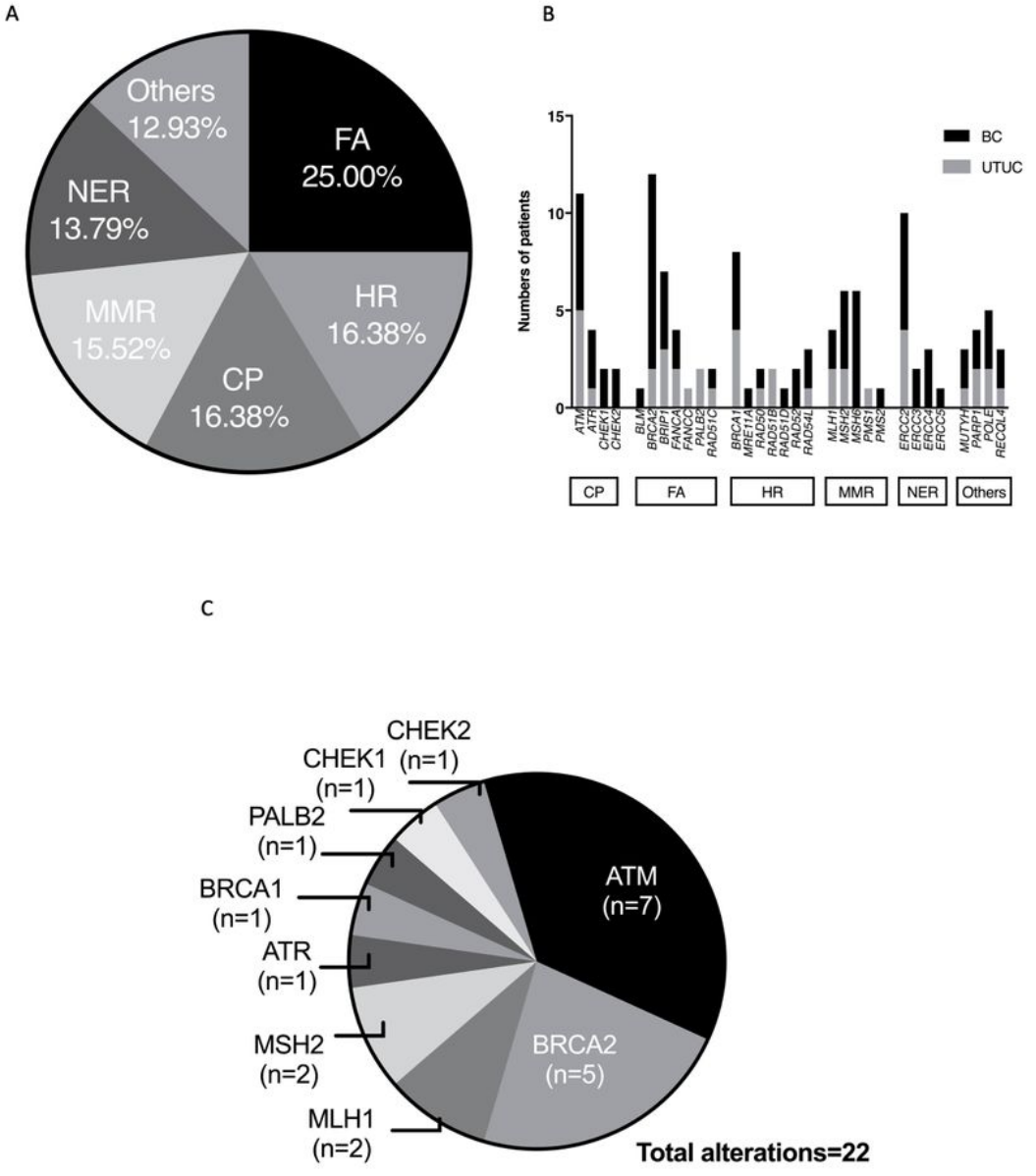



## Figure 3

Genomic features of FGFR and ERBB in UC patients. (A) Oncoprint illustration of somatic alterations in FGFR genes (FGFR1, FGFG2, FGFR3 and FGFR4). (B) Alterations of FGFR2 and FGFR3 genes indicated as

amino acids changes. (C) Oncoprint illustration of somatic alterations in ERBB genes (EGFR, ERBB2 and ERBB3). (D) Alterations of ERBB2 gene indicated as amino acids changes. Red: amplification; Blue: inframe insertion or deletion; Green: Missense; Black: Truncating.

Figure 4

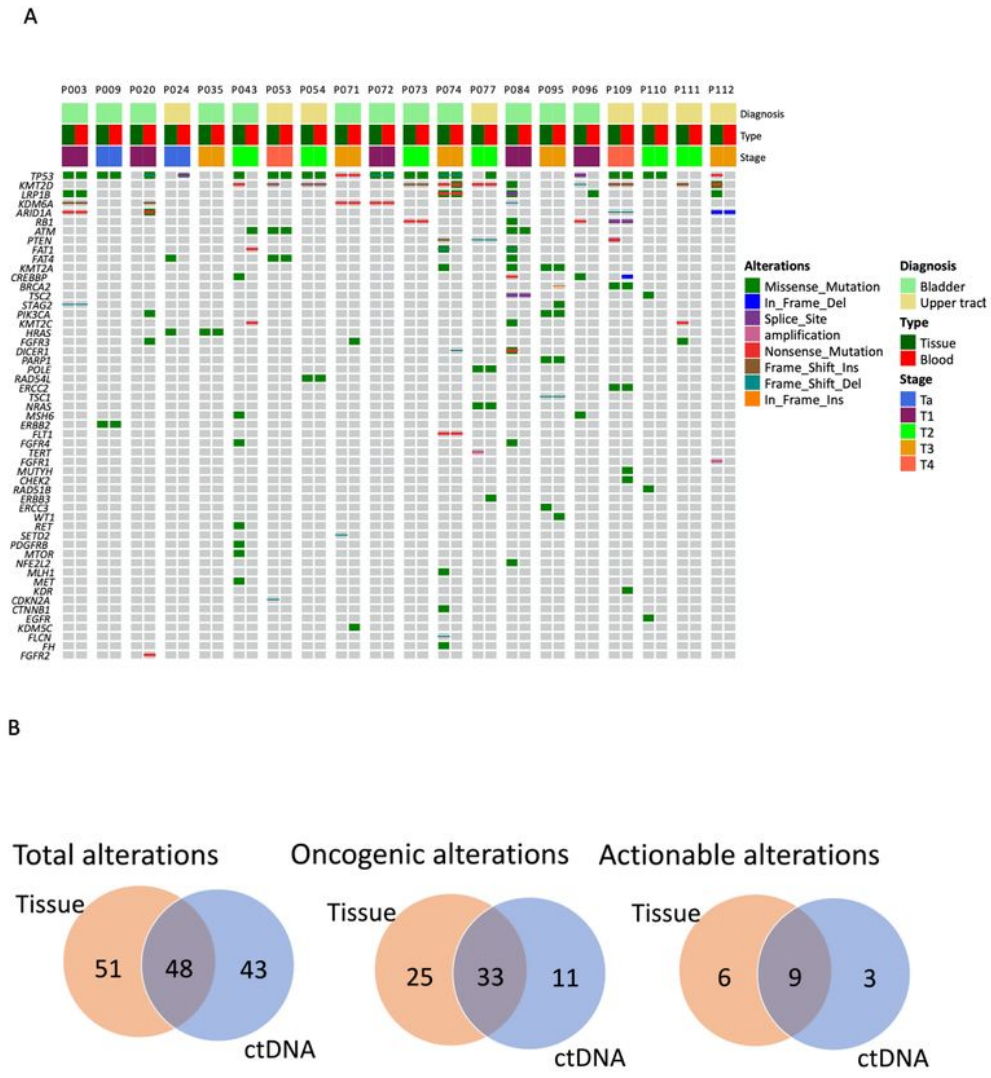



## Figure 4

Somatic alterations in DDR pathway. (A) Frequency of altered pathway for DDR. (B) The distribution of somatic alterations in specific DDR gene of UTUC and UCB samples. (C) The distribution of known or

likely deleterious somatic DDR gene mutations. DDR: DNA damage repair; UCB: bladder carcinoma; UTUC: upper tract urothelial carcinoma; FA: Fanconi anemia; HR: homologous recombination repair; CP: checkpoint; MMR: mismatch repair; NER: nucleotide excision repair.

Figure 5




## Figure 5

Concordance of genetic alterations between ctDNA in serum and matched tumor tissue. (A) Oncoprint illustrating of the similarities and differences between matched serum and tumor collections. (B) Venn

diagram showed the number of total mutations, oncogenic mutations and actionable mutations in total matched serum and tumor samples.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementalTable2Genelist.xlsx
- SupplementalTable1Patientsclinicalcharacteristics.xlsx
- SupplementalTable3Germlinevariants.xlsx