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Review

The emerging role of somatic tumor sequencing in the treatment of urothelial cancer

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Abstract The development of rapid genome sequencing has greatly enhanced our understanding of the molecular biology underlying many malignancies. Whole exome sequencing has highlighted the individualistic nature of malignancies on a patient-to-patient basis and begun to revolutionize therapeutic approaches. In recent years, whole genome sequencing of urothelial malignancies has identified a host of somatic mutations which contribute to growth, progression, and metastasis of urothelial carcinoma of the bladder and upper tract urothelial carcinoma. As genetic sequencing continues, additional targets will be identified, allowing development of novel therapeutic agents targeting cancer on a molecular level, with the goal of delivering highly individualized care based on the underlying mutational profile of the patient's malignancy. In this review, we aim to discuss known genetic alterations of urothelial malignancy and the implications these mutations carry in terms of prognostication and development of targeted therapeutic agents. We will focus on RNA-expression profiling and genomic DNA profiling, with a focus on comprehensive whole exome and whole genome sequencing relative to selected urothelial carcinoma-associated genes and circulating tumor DNA analysis.

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1. Introduction

Cancer can arise from a broad variety of mutations within our genomic makeup. These molecular changes may be inherited, caused by environmental factors, and/or accumulate over time. Advances in DNA sequencing over the last decade have allowed for the identification of a myriad of mutations from the human genome [1,2]. Through these endeavors, it has been hypothesized that we have uncovered the most common pathogenic genetic aberrations across 21 cancer types [3]. Paradoxically, as more knowledge has been gained regarding the genomic basis of cancer, it has become evident that much more research is needed to further understand its complexity.

Somatic mutations arise through the accumulation of replicative error over time, with or without attributable environmental factors [4]. This contrasts with germline mutations which are predominantly inherited. There are well described germline mutations in genitourinary cancers, with current National Comprehensive Cancer Network (NCCN) guidelines recommending screening for familial mutations in patients with strong family history or particularly aggressive disease with the presence of several malignancies [5,6]. Once a malignancy is identified, it can be difficult to attribute etiology purely to somatic versus germline mutations. In these cases, simultaneous sequencing tumor and clinically normal adjacent tissue can help delineate the underlying drivers of cancer [7].

Understanding the genomic determinants of malignancy has many important clinical implications. First, improved recognition of tumor characteristics has the potential to predict treatment outcome and guide therapy. Most importantly, it may facilitate identification of novel targets for therapy. In the following review, we will discuss recent advances in somatic tumor sequencing in urothelial cancer.

2. Advancements in genetic sequencing

Genomic changes resulting in malignancy can occur from copy number alterations, changes in protein expression, protein structural changes, regulatory derangements, and epigenomic variations [7]. The tools utilized to understand these somatic drivers of cancer have evolved over time. While the Human Genome Project (HGP) took 13 years and cost billions of dollars in funding to sequence the human genome, today a complete genomic sequence can be performed in a matter of days, costing less than $1000 [8,9].

Next-generation sequence (NGS) is the process of efficiently decoding the genome by simultaneous sequencing of many small fragments of DNA at the same time. Whole-exome sequencing (WES) is focused on protein coding regions of DNA. While WES has allowed for the characterization of many tumor drivers and has reshaped the field of oncology, somatic mutations within non-coding regions which make up more than 98% of the human genome remain largely underexplored [7]. In contrast, whole genome sequencing (WGS) covers numerous regulatory regions, noncoding RNA, and structural motifs. In a landmark decision by the Center of Medicare and Medicaid Services (CMS), the Food and Drug Administration (FDA) approved NGS testing which became covered under Medicare for patients

with advanced cancers [10]. Today, commercially available germline, somatic, and circulating cell free DNA testing are readily available to clinicians and patients [11].

3. Genomic sequencing in bladder cancer

Bladder cancer tumor staging is based on depth of tumor invasion. The distinction between non-muscle invasive bladder cancer (NMIBC) and muscle invasive bladder cancer (MIBC) has clinical and prognostic implications. NMIBC recurs frequently, with approximately 50% of patients experiencing at least one recurrence at a median follow-up of 4 years [12]. While the majority of NMIBC can be managed endoscopically with transurethral resection with or without intravesical therapies, some patients, particularly those with high-risk NMIBC can progress to muscle invasion. MIBC is associated with poor prognosis and high mortality despite advances with treatment. In fact, the five-year survival rate remains 60% for localized disease, with less than 10% in the setting of metastases [13].

Bladder cancer is characterized by one of the highest somatic mutation loads of all human cancers, especially with respect to MIBC [14,15]. Our current understanding of the genomic makeup of MIBC was improved by the seminal findings published by The Cancer Genome Atlas (TCGA) in 2014. Since its conception in 2006 under the collaborative effort of the National Cancer Institute (NCI) and the National Human Genome Research Institute (NHGRI), the TCGA has characterized the genomic, transcriptomic, and epigenetic alterations of 33 cancer types to date [16]. In 2014, the landmark TCGA-2014 study was published detailing the molecular alterations commonly encountered in MIBC [14]. By extracting WES data from 131 chemotherapy-naive, high grade MIBC specimens and tissue matched normal tumor-adjacent tissue samples or blood, 32 statistically significant recurrent genetic alterations in MIBC were described. In 2017, data from an additional 412 samples were analyzed to identify additional mutations (TCGA-2017) [17]. In addition to single gene mutations, a combination of specific sequences of mutations can occur in a predictive pattern. Analysis of these "mutational signatures" from the Catalogue of Somatic Mutations in Cancer (COSMIC) has been compiled, primarily from WES techniques, and can yield further diagnostic, prognostic, and therapeutic targets [18]. Below, we describe the major genomic mutations noted from the TCGA and other datasets.

3.1. *TP53*

The loss of tumor suppressor genes is a common inciting event in tumor initiation. The *TP53* gene is the single most commonly mutated gene found in human cancers and encodes for tumor suppressor p53 [19]. In TCGA-2017, *TP53* was altered in 48% of samples and was the most common somatic mutation identified. Additionally, the associated p53 cell signaling pathway was found to be inactivated in 89% of all MIBC specimens analyzed. Interestingly, a prior meta-analysis of 117 studies had found that *TP53* mutation is only weakly predictive for bladder cancer recurrence, progression, and mortality [20]. The limited prognostic

utility of *TP53* was confirmed in TCGA-2017. Another commonly deregulated tumor suppressor gene in bladder cancer is *RB1*, which occurs in 17% of MIBC specimens, and has been shown to predict cisplatin-based neoadjuvant chemotherapy (NAC) responsiveness [21]. Losses of activity of the *TP53* and *RB1* pathways are attractive targets for novel drug development.

3.2. Chromatin-modifying genes

Bladder cancer is characterized by high rates of mutations within chromatin-modifying or chromatin-regulatory genes (44%). Key members of this family include *KMT2D*, *KDM6A*, and *ARID1A*, which account for the second, third, and fourth most commonly observed single gene mutations from TCGA-2017. *KMT2D* (28%) encodes for a histone methyltransferase; *KDM6A* (26%) encodes a histone demethylase; and *ARID1A* (25%) encodes for a key component of the chromatin remodeling complex. Within *in vitro* models, *KDM6A*-null bladder cancer cell lines were sensitive to EZH2 inhibition, making it an attractive therapeutic target for further investigations [22].

3.3. *RTK-RAS-PI3K* pathway

Deregulation of the *RTK-RAS-PI3K* pathway is also common in MIBC. Fibroblast growth factor receptor-3 (*FGFR3*) is a key regulator of cell cycle entry and proliferation, and its role in NMIBC has been increasingly studied [23]. Mutations in *FGFR3* are associated with low-grade disease and are generally associated with good prognosis in NMIBC [24]. Even with respect to MIBC, the presence of *FGFR3* alterations were seen in 14% of cases, and were predictive of lower stage tumors and better survival [17]. There have been several recently approved agents targeting *FGFR3* in the metastatic setting including infigratinib (BGJ398) and erdafitinib, which recently was granted FDA-approval for patients with *FGFR2* or *FGFR3* mutations [25,26]. *ERBB3* is another commonly mutated gene in this pathway. It encodes for human epidermal growth factor receptor 2 (Her2) and is amplified in 12% of cases. Her2 overexpression is associated with variant micropapillary histology and overall poor prognosis [27]. In addition to traditional point mutations, products of chromosomal rearrangements are being increasingly recognized in oncogenesis. A prominent example of such is the *FGFR3-TACC3* fusion mRNA product that results in autodimerization and constitutive activation of the FGFR3 kinase domain [14]. Another example is ERBB2, where fusion with DIP2B promotor results in DIP2B-ERBB2 overexpression. These fusion proteins are potential attractive targets for novel treatment developments.

3.4. *PI3K/AKT/mTOR* pathway

Deregulation of this pathway is seen in 42% of MIBC tumors, and has potential therapeutic implications [14]. Select patients with mutations in this pathway have shown response to investigational mTOR inhibitor treatment [28,29]. It is worth noting that responses are not uniform for all patients and warrants further investigation.

3.5. DNA damage repair (DDR)

Commonly mutated genes from this class observed in bladder cancer include *ERCC2*, *FANCC*, and *ATM*. *ERCC2* plays a key role in nucleotide excision repair and is altered in 9% of MIBC. Numerous publications have demonstrated that mutations in this class of genes are associated with improved responsiveness to cisplatin-based chemotherapy [21,30,31].

3.6. The apolipoprotein B mRNA editing enzyme catalytic polypeptide-like (APOBEC) family

The APOBEC family is comprised of proteins responsible for DNA single strand editing. Mutations in APOBEC are well described in bladder cancer [32]. Tumors with high APOBEC expression have significantly higher mutations in *TP53*/*RB1*, DNA damage response, and chromatin modifying genes, with significantly improved overall survival as compared to APOBEC-low tumors [33].

3.7. COSMIC 5

Smoking is a well-studied risk factor for bladder cancer initiation, recurrence, progression, and mortality in bladder cancer [34]. COSMIC 5 is a mutation signature that is enriched in smokers in bladder cancer [35].

3.8. COSMIC 22

Aristolochic acid is a mutagen associated with development of urothelial carcinoma. COSMIC 22 is a mutation signature identified in upper tract urothelial carcinoma with exposures to aristolochic acid [35].

4. MIBC molecular subtypes

Molecular subtyping aims to group tumors together based on shared characteristics such as DNA sequencing, gene expression, and epigenetic features. The goal of such grouping schemes is to improve assessments of prognosis and treatment outcomes to ultimately facilitate targeted treatments for individual patients. This is particularly desirable in a cancer with high frequency of mutation and genomic variation across tumors. Several prominent groups have published different classifications to group MIBC based on molecular findings. The TCGA-2014 publication first categorized MIBC tumors based on their mRNA expression profiles, resulting in four distinct clusters. Cluster I and II overlapped in terms of luminal characteristics and markers. Cluster III is a predominately basal/squamous-like subtype that is enriched for stem cell features, and Cluster IV had epithelial-mesenchymal features. The TCGA-2017 publication re-classified MIBC into five molecularly distinct categories based on mRNA expression: Luminal-papillary (35%), luminal-infiltrated (19%), luminal (6%), basal-squamous (35%), and neuronal (5%). In this classification, the luminal-papillary subtype, characterized by *FGFR3* mutations classically associated with NMIBC, conferred the best overall survival. The basal-squamous

subtype had a strong female predominance and association with carcinoma *in situ*. The least common neuronal subtype expresses neuroendocrine signatures and was associated with the worst clinical outcomes. Several other groups have also independently put forth molecular classification schemes. The UNC classification divided 262 high-grade MIBC samples into two subtypes, basal and luminal [36]. The MD Anderson group added a third subtype based on enrichment for expression of *TP53* related genes [37]. The Lund molecular taxonomy was derived from tissue comprised of both MIBC as well as NMIBC tumors, and categorized the samples into molecular subtype (MS)1 and MS2 based on gene expression profiling [38,39]. The Baylor classification produced an 18-gene signature which was applied to the TCGA RNA-seq profile to characterize urothelial differentiation [40]. The Cit-Curie transcriptomic classifier was built from clustering of seven independent datasets of MIBC tumors and proposed a "basal-like" subtype driven by *EGFR* mutations [41]. In general, tumors with basal features appear to have more aggressive features (*i.e.* TCGA Cluster III, UNCA basal-like subtype, and Lund uroB subtype).

In 2019, an effort was made to reach an international consensus on MIBC molecular subtypes to provide a more cohesive framework for future work in the field. By analyzing and combining features of the six published classifications systems discussed above, Kamoun et al. [13] reported six molecular classes of MIBC.

4.1. Basal/squamous (35%)

The most common subtype of MIBC identified is characterized by *TP53/RB1* mutations, as well as 3p14.2 deletion. It was correlated with the basal-squamous subtype from TCGA-2017 in terms of female predominance, higher clinical stage, and poor prognosis, with median survival of 1.2 years.

4.2. Luminal papillary (24%)

This second most common subtype is characterized by *FGFR3*, *KDM6A*, and *CDKN2A* mutations. It was more common in patients younger than 60 years of age and was associated with the best prognosis of described subtypes, with median survival of 4 years.

4.3. Luminal unstable (15%)

This subtype derived its name from possession of the most genetic alterations. Commonly mutated genes include *PPARG*, *E2F2*, *SOX4*, *ERBB2*, *TP53*, and *ERCC2*. It was associated with median survival of 2.9 years.

4.4. Luminal nonspecified (8%)

This subtype was commonly described in older patients (age >60 years), and enriched for antigen presenting genes as well as immune checkpoint markers. It was associated with poor prognosis with median survival of 1.8 years.

4.5. Stroma-rich (15%)

This subtype was characterized by predominance of smooth muscle, fibroblast, and myoblast features. It was associated with good prognosis with median survival of 3.8 years.

4.6. Neuroendocrine-like (3%)

This was the rarest of all described subtypes, with high rates of concurrent *TP53* and *RB1* inactivation. It had the worst prognosis out of all subtypes, with median survival of 1 year.

5. NMIBC molecular subtypes

In 2016, transcriptomic analysis of a European multicenter database of 460 NMIBC specimens allowed for molecular classification of NMIBC [42]. From analysis of total RNA sequencing data, tumors were stratified into three classes based on gene expression profiles that partly aligned with the previously described Lund taxonomy system [38]. Class 1 was comprised of luminal-like tumors that expressed early cell cycle regulating genes and had an overall good prognosis. Class 2 was comprised of high risk luminal-like tumors that expressed genes potentially involved in progression from CIS and associated with poor prognosis. Meanwhile, Class 3 was comprised of basal-like tumors with higher levels of tumor suppressor expression, corresponding with low tumor cell-cycle and metabolic activities. In general, high grade NMIBC tumor shared features of MIBC and were more likely to exhibit mutations in *ERBB2*, *p53/RB1*, *CDM6A*, and *ARID1A*. These genes are involved in critical regulatory pathways such as DNA repair, cell cycle regulation, and chromatin modification. Low grade NMIBC was characterized by distinct subset of mutated genes which include *FGFR3*, *STAG2*, and *PIK3CA*. *TERT* promoter region mutations have been identified across the spectrum of NMIBC risk groups [43]. These findings are critical to augment our current risk stratification of NMIBC to tailor management, particularly regarding the feasibility and appropriateness of bladder sparing approaches.

6. Impact of tumor sequencing on MIBC treatment

The gold standard treatment for MIBC is NAC followed by radical cystectomy, or in some patients, chemoradiation [44]. In recent years, systemic immunotherapy has become an additional potential neoadjuvant treatment option for patients. A well-known limitation of these approaches is an inability to predict response. Radical cystectomy is characterized by high perioperative mortality of 1.2%—3.2% at 30 days, which has been reported to be as high as 8% at 90 days post-operative [44]. The ability to predict response to neoadjuvant therapy has multiple benefits including: Tailoring appropriate neoadjuvant therapy, understanding when to suspend or switch neoadjuvant treatments, and potentially avoiding definitive surgical or radiation treatments after neoadjuvant treatments are completed [45,46].

7. Predicting neoadjuvant chemotherapy response

Level 1 evidence has demonstrated that cisplatin-based NAC confers at least an absolute 5% overall survival benefit when utilized for cT2-4N0M0 cancers [47,48], but is limited by its extensive side effect profile which includes cardiac, vascular, hearing, neuronal, and renal toxicity [49]. While data have shown increasing adoption of NAC, prior studies have demonstrated that only 17%–30% of MIBC ultimately receive NAC [50,51]. Data from the SEER-Medicare database have shown that this number may be as low as 1.4%–11% in the community setting [52]. There is established variation in response to NAC. Somatic tumor sequencing may help to identify which patients may benefit from receiving NAC versus proceeding directly to upfront surgery.

From a tumor sequencing perspective, MIBC with mutations involving DDR and cell cycle regulation have demonstrated increased sensitivity to platinum-based NAC. A prospective study comparing pre- and post-systemic accelerated methotrexate, vinblastine, doxorubicin, and cisplatin (AMVAC) treated tumor samples identified one or more mutations in DDR pathways (*ATM, RB1,* and *FANCC*) in 87% of patients who experienced pathologic response [21]. Perhaps more interestingly, none of the non-responders harbored mutations in these genes, suggesting that DDR mutations in bladder cancer may prime patients to NAC response. At follow-up, patients with DDR mutations had an improved 5-year survival rate of 85%, which is nearly double that of patients with no DDR mutations at 45% [53]. Separately, WES analysis of patients with bladder cancer who underwent NAC followed by radical cystectomy using Memorial Sloan Kettering Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) showed that patients with DDR alterations have improved progression-free survival and overall survival [54]. This finding is congruent with our current understanding of the therapeutic mechanism of action of cisplatin, which effectively triggers cancer cell death by inducing DNA damage by crosslinking, leading to apoptosis. In this way, bladder tumors with existing mutations in DDR pathways may be more sensitive to cytotoxic effects of platinum. Based on these findings, large scale phase 2 clinical trials are underway to explore therapeutic applications of DDR mutations in bladder cancer. Among these include risk-adapted treatment approaches based on tumor sequencing (RETAIN, NCT02710734), neoadjuvant ddGC (dose dense gemcitabine and cisplatin) in patients with DDR mutations (A031701, NCT03609216), and combination neoadjuvant nivolumab, gemcitabine, and cisplatin for MIBC with assessment of response using genomic biomarkers (GU16-257, NCT03558087).

Within the DDR cohort, the best example of a single gene mutation which predicts NAC response is *ERCC2*, a nucleotide excision repair gene commonly mutated in MIBC. NAC responsive patients are found to have tumors enriched for mutations in *ERCC2* [30,55]. Although these preliminary data are encouraging, *ERCC2* status did not appear to prognosticate survival in the TCGA cohorts [14,30]. Additionally, patients with expression of *FGFR3*, a gene which predominantly characterizes NMIBC and less aggressive MIBC, have improved survival [42]. Some groups have also attempted to develop genetic profiling tools to assess for NAC responsiveness, but small sample sizes have limited clinical applicability [56,57].

One goal of molecular subtyping is to characterize tumors for improved prognostication. In the NAC-free subgroup for the TCGA cohort, the tumors with basal features had worse outcome comparatively to the luminal types. From the 2019 international consensus subtypes, tumors with basal-squamous or luminal non-specified tumors have improved survival after receiving NAC [13]. Based on a prior publication, the absence of p53 alteration suggested correlation with NAC response [58]. From this, it was hypothesized that expression of the p53 tumor suppressor is chemoprotective in the setting of NAC by guarding against DNA damage. This observation is recapitulated in the MD Anderson classification dataset; tumors within the p53-like subtype did not respond to NAC [37]. This finding was further supported by a randomized trial of 60 patients treated with cisplatin-based NAC prior to surgery [59]. It is important to point out that overall, p53 status along is not predictive of treatment response or survival outcome.

8. Predicting immunotherapy response

Following recent successful implementation of immune checkpoint inhibitors in the management of cisplatin-treatment refractory metastatic urothelial carcinoma, there has been subsequent interest in moving its utilization earlier to the neoadjuvant setting [60]. One of the agents of interest is pembrolizumab, a humanized antibody which binds and blocks programed cell death ligand (PD-L1) on lymphocytes, triggering the immune system to target and destroy cancer cells. In 2018, Necchi et al. [61] reported the results of the PURE-01 study in which patients with cT2-3N0M0 bladder cancer who were treated with neoadjuvant pembrolizumab exhibited complete pathologic response of pT0 in 42% of subjects. This group followed up with the report of RNA-based immune signature scores which may predict both response and progression free survival after neoadjuvant pembrolizumab [62]. From the TCGA-2014 dataset, the cluster II luminal subtype exhibited resistance to NAC, but demonstrated exquisite sensitivity to atezolizumab, another PD-L1 inhibitor [37,63]. Following this, a single-arm ABACUS trial demonstrated complete pathologic response in 31% of patients treated with neoadjuvant atezolizumab [64]. More work is needed to evaluate the role of molecular subtyping in stratifying patients into either neoadjuvant immunotherapy or NAC based on expected response. Several emerging predictors of therapeutic response have been summarized in Table 1.

9. Upper tract urothelial carcinoma

While upper tract urothelial carcinoma (UTUC) and urothelial cancer of the bladder (UCB) were historically considered identical, the advent and proliferation of NGS and WES have shifted our understanding of their distinct molecular biologies. In 2015, the first large scale NGS study focusing on UTUC was published by Sfakianos and colleagues [65]. Following this publication, several subsequent studies examining WGS have been published, refining our

Table 1   Predictors of response to neoadjuvant bladder cancer therapies prior to cystectomy in MIBC.

Marker	Function	Predictive utility	Neoadjuvant regimen
DDR proteins	Cell cycle regulations	Enrichment predicts response	AMVAC [21]
ERCC2	Nucleotide excision repair gene	Enrichment predicts response	GC, ddMVAC, GC-Sunitinib, ddGC [30]; AMVAC, ddGC [55]
FGFR3	Fibroblast growth receptor	Enrichment improves survival	Not applicable [42]
P53	Tumor suppressor	Absence of alteration predicts response	MVAC, PAC, EP [58]; ddMVAC [59]
Immune190 signature, IFN gamma, and IFN alpha	Biomarkers	Enrichment predicts response	Pembrolizumab [62]
pCR and MPR	Biomarkers	Enrichment predicts response	Atezolizumab [64]

DDR, DNA damage response; AMVAC, accelerated methotrexate, vinblastine, doxorubicin, and cisplatin; GC, gemcitabine and cisplatin; ddMVAC, dose-dense methotrexate, vinblastine, doxorubicin, and cisplatin; ddGC, dose-dense gemcitabine and cisplatin; PAC, Cisplatin, Doxorubicin, Cyclophosphamide; EP, Etoposide, Cisplatin; IFN, interferon; pCR, pathologic complete response; MPR, major pathologic response.

understanding of the molecular biology underlying UTUC [65–67]. While UTUC and UCB share common somatic genomic alterations, the frequency of mutated oncogenes and tumor suppressors differs considerably [66]. More comprehensive understanding of the differences in molecular biology between the two entities will ultimately yield molecular pathways for targeted therapeutic agents, and clinical trials are currently underway seeking to take advantage of known genetic mutations.

Frequent genetic mutations in UTUC include *FGFR3*, chromatin remodeling genes (*KMT2D, KDM6A*), *TP53/MDM2*, and other commonly mutated tumor suppressors and oncogenes (*CDKN2A* and *RAS*) [66]. While many genetic alterations are found commonly in UCB and UTUC, the frequency of these mutations varies considerably. The largest study comparing genomic profiles between UTUC and UCB reported significant differences in mutational profiles [68]. *FGFR3* and *HRAS* are mutated at higher rates in UTUC, while lower mutational frequency of *TP53*, *RB1*, and *ERBB2* are noted when compared to UCB [68]. In addition to differences in the overall molecular landscape of UTUC and UCB, whole exome sequencing demonstrated differences in high-grade UTUC and UCB. High-grade UTUC is more commonly affected by mutations in *FGFR3*, *HRAS*, and *CDKN2B*, while mutations in *TP53*, *RB1*, and *ARID1A* were more likely associated with high-grade UCB [65]. Differences are also seen in the molecular profiles of high-grade and low-grade UTUC. Low-grade tumors more frequently exhibit mutations in *FGFR3*, while *TP53* mutations are characteristic of high-grade UTUC and confer greater genomic instability. The study by Audenet and colleagues [68] also found UTUC with bladder recurrence was correlated with certain genomic alterations, notably mutations in *FGFR3*, *KDM6A*, and *CCND1*. The same study found *TP53* appeared to confer lower risk of bladder recurrence.

Similar to the six molecular classes of MIBC, recent comprehensive genomic analysis of UTUC produced four distinct molecular-clinicopathologic classifications, which differ in terms of luminal versus basal subtype, frequency

of *FGFR3* mutations, bladder recurrence, muscle-invasive disease, and survival [14,66]. Molecular cluster 4 has been shown to have higher mRNA expression of *CTLA4*, *CD274* (*PDL1*), and *PDCD1* (*PD1*), suggesting common immunotherapy agents such as nivolumab, pembrolizumab, and ipilimumab may yield greater clinical outcomes in metastatic UTUC with certain genetic profiles [67].

With recent focus on NGS, novel therapeutic targets have been discovered for targeted therapy. While WGS for upper tract urothelial malignancy is in its infancy, several promising targets have been identified for potential systemic therapy, most notably the *FGFR3* pathway. One therapeutic agent, infigratinib, has been trialed in small cohorts of patients with metastatic urothelial malignancy and activating *FGFR3* mutations. One such study by Pal and colleagues [69], enrolled 59 UCB and eight UTUC patients with metastatic disease. While the cohort was small, objective response rates differed significantly between the groups, with a 50% objective response rate in UTUC and 22% in UCB. Other studies are also underway with *FGFR* inhibitors, notably erdafitinib and pemigatinib [25,61,70]. While *FGFR3* mutations are found more frequently in low-grade, noninvasive disease, studies have reported that up to 60% of high-grade UTUC have activating *FGFR3* mutations.

10. Conclusions and future directions

While early results from genome wide sequencing have yielded molecular pathways for targeted therapy, our understanding of the molecular biology requires further work. First, validation is required, particularly for the optimal genomic and molecular subtype schemes to use in clinical practice. Furthermore, a vast amount of genetic heterogeneity exists within individual bladder tumors, which poses practical problems in utilization of somatic tumor sequencing [14]. Further study will likely discover new genomic alterations that can be taken advantage of by using immunotherapy and chemotherapy agents. With

increased research and evaluation, we expect that tumor sequencing will have an impact on the clinical management of urothelial carcinoma.

Author contributions

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Conflicts of interest

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