Review Article on Update on Molecular Classification and Individualized Treatments of Genitourinary Tumors

TNM staging towards a personalized approach in metastatic urothelial carcinoma: what will the future be like?—a narrative review

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Abstract: The American Joint Committee of Cancer (AJCC) tumor-node-metastasis (TNM) classification, with its periodical updates and modifications, has represented and still represents the basis of cancer staging. The historical, long-standing limitations of anatomic-based TNM staging have been recently "threatened" by the impressive amount of data derived from molecular analyses, which have led to an unprecedented level of understanding of cancer genomics. In fact, current era of personalized oncology has witnessed important efforts towards the integration between clinical, anatomical and molecular features; however, despite the promises, personalized oncology faces many obstacles, due to the complex relationship between tumor biomarkers, previously unknown cancer subtypes and clinical and anatomical characteristics. With regard to urothelial carcinoma (UC), the characterization of tumors in large cohorts of patients has provided important information concerning genetic alterations, revealing the presence of biologically relevant subtypes of UC. In the current review, we will provide an overview regarding this recent "translation" from the anatomic-based TNM to a novel horizon, aiming at further "tailoring" personalized oncology, especially focusing on recently published data about the molecular landscape of UC with its therapeutic and prognostic implications.

Keywords: Metastatic urothelial carcinoma; tumor-node-metastasis (TNM); bladder cancer; muscle-invasive bladder cancer (MIBC); gene signatures; genomic subtypes

Submitted Jul 22, 2020. Accepted for publication Aug 18, 2020.
doi: 10.21037/tau-20-1109
View this article at: http://dx.doi.org/10.21037/tau-20-1109

Introduction

Urothelial carcinoma (UC) is among the most common malignancies worldwide, with around 450,000 new diagnoses each year (1). To date, UC represents the eleventh most frequently diagnosed malignancy in both sexes and the seventh in the male population throughout the world (2). The most important risk factor is tobacco smoking, which is held responsible for the 50% of all UCs, followed by pelvic radiation, occupational exposure to carcinogens and genetic predisposition (3). Overall, about three quarters of UC patients are diagnosed with non-muscle-invasive bladder cancer and are treated with transurethral resection and intravesical instillation of Bacillus of Calmette-Guerin (BCG) or other anticancer agents (4,5). Unfortunately, the remaining 25% of patients presents with muscle-invasive bladder cancer (MIBC)—classically defined by the invasion of the detrusor muscle—or metastatic disease (6). Moreover, approximately the 50% of patients with tumor stages among T2b and T4a develop metastatic disease following radical surgery (*Figure 1*) (7). Despite platinum-based chemotherapy is the backbone of treatment for metastatic UC, with the combination of cisplatin plus gemcitabine or methotrexate, vinblastine, doxorubicin and cisplatin (M-VAC) (8,9), an important percentage of patients is unfit to receive cisplatin because of comorbidities, old age, peripheral neuropathy and/or poor Eastern Cooperative Oncology Group performance status (ECOG-PS) (10).

The last decade has witnessed notable advances in UC management and previous treatment paradigms of metastatic disease have been modified by immune checkpoint inhibitors (ICIs), which have rapidly emerged as novel therapeutic options (11). In fact, although platinum-based regimens remain the standard first-line treatment for cisplatin-eligible advanced UC patients, therapeutic options are dramatically evolving with the US Food and Drug Administration (FDA) approval of second-line pembrolizumab, nivolumab, avelumab, atezolizumab and durvalumab (12). Moreover, the therapeutic algorithm of metastatic UC is further evolving, with the results of the JAVELIN Bladder 100 trial which have been recently presented at the virtual 2020 ASCO Annual Meeting (13). This phase III trial comparing maintenance avelumab versus best supportive care in UC patients who achieved stable disease, partial response or complete response from first-line platinum-based chemotherapy, has reported a significant improvement in overall survival (OS) in the avelumab arm (21.4 versus 14.3 months; HR, 0.69; 95% CI, 0.56–0.86; P<0.001). Thus, first-line maintenance therapy with the anti-PD-L1 avelumab is destined to become a new standard of care in patients with advanced UC achieving disease control with first-line platinum-based chemotherapy. Nonetheless, several issues remain since the prognosis of patients affected by metastatic disease is still dismal, with a 5-year OS of around 10% (14).

If the American Joint Committee of Cancer (AJCC) tumor-node-metastasis (TNM) classification has among its purposes to properly define cancer staging, the identification of different UC molecular features has allowed to integrate the TNM model with brand-new elements (*Table 1*) (15,16). In fact, the integration between TNM anatomic-based characteristics, baseline clinical features and the molecular landscape of UC has led to a novel, personalized paradigm in cancer management (17). From a molecular point of view, UC resulted to be a heterogeneous disease, with high mutational rate and genomic instability. In fact, UC is characterized by a marked inter-tumoral and intra-tumoral heterogeneity, which have contributed to the lack of effective targeted treatments in early studies (18). In the last decade, the molecular landscape of UC has begun to emerge, offering the possibility to unveil the basis of UC carcinogenesis and tumor progression (19); moreover, molecular profiling of UCs has become increasingly meaningful due to the identification of potentially targetable molecular alterations, including Fibroblast Growth Factor Receptor (FGFR), Human Epidermal Growth Factor Receptors and DNA damage response (DDR) pathway (20,21). In fact, multiplatform genomic profiling has paved the way towards a new era in UC management, where biomarker-driven clinical trials appear as a mandatory need.

In the current review, we discuss recent advances regarding the characterization of UC, having the potential to integrate the TNM classification with molecular subtyping in this aggressive disease. A comprehensive literature search on PubMed/Medline, Cochrane library and Scopus has been performed using the keywords “bladder cancer” OR “bladder carcinoma” OR “urothelial carcinoma” OR “muscle-invasive bladder cancer” AND “gene signatures” OR “TCGA” OR “genomic subtypes”. We selected the most relevant and pertinent reports on the basis of the quality of the studies in terms of their applicability, how they were conducted and the number of patients included. Despite molecular profiling studies have better defined the genetic landscape of UC, suggesting the presence of different patterns of mutations, advanced/

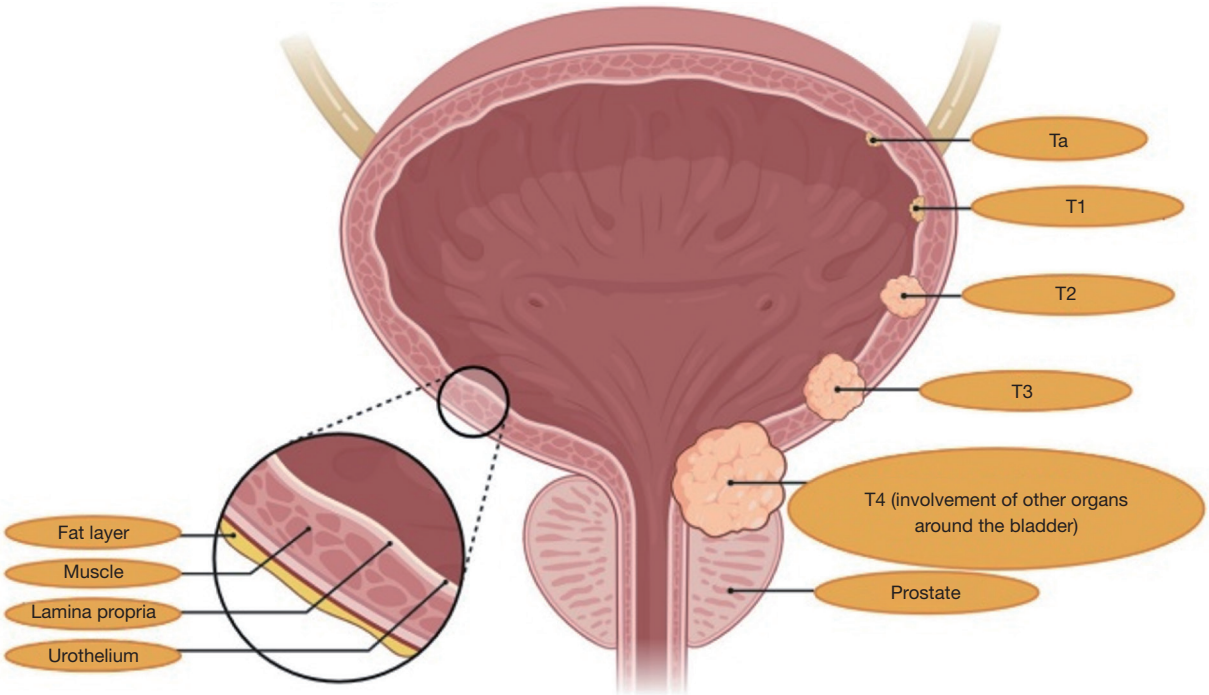

Figure 1 Figure showing some of the T stages of bladder cancer. Ta: the cancer is in the innermost layer of the bladder lining; T1: the cancer has started to grow into the connective tissue beneath the bladder lining; T2: the cancer has grown through the connective tissue into the muscle; T3: the cancer has grown through the muscle into the fat layer; T4: the cancer has spread outside the bladder.

metastatic UC remains a complex, difficult-to-treat malignancy and more work is warranted in the near future in this direction. We present the following article in accordance with the NARRATIVE REVIEW reporting checklist (available at http://dx.doi.org/10.21037/tau-20-1109).

NGS and baseline characteristics in clinical practice: what we should remember

After the completion of the Human Genome Project in 2003, sequencing of cancer genomes has represented one of the hottest topics in cancer research, with a view to led to a better comprehension of the genetic basis of oncogenesis and tumor progression (22). Concurrently, next-generation sequencing (NGS) technology has incredibly expanded with notable advances in terms of reliability, data interpretation and costs, making NGS feasible in everyday clinical practice (23,24). Before NGS, molecular tumor alterations were identified using single gene assays; conversely, NGS technology has allowed to perform simultaneous analyses of hundreds of genes through targeted sequencing panels (25). In fact, NGS has allowed a faster and simpler sequencing, improving clinicians and researchers understanding of cancer and promoting the birth of a new era, that of precision medicine—and precision oncology (26). One of the aims of precision oncology is to tailor oncological treatments to the single patient's characteristics, on the basis of a deep characterization, the identification of druggable mutations and the presence of specific biomarkers (27). And importantly, the use of NGS has created the basis for a new horizon, moving from an anatomical and clinical "stratification"—according to clinical, anatomical and pathological features—to a more personalized approach (28).

As we shall see later, UC is a heterogenous, complex disease including several tumor subtypes presenting important and emerging differences, which have been only partially identified. In the efforts towards precision oncology, NGS is a golden tool which is assuming and will assume an increasingly important role; in fact, incorporating genomic information in the diagnostic and staging processes is one of the current and future challenges in UC management, a crucial step in order to improve clinical outcomes in this

Table 1 American Joint Committee—TNM stage classification of bladder cancer

Stage	Stage grouping	Stage description
0a	Ta	Non-invasive papillary carcinoma (Ta) with no nodal involvement (N0) or distant sites (M0)
	N0	
	M0	
0is	Tis	Flat, non-invasive carcinoma (Tis)—also known as flat carcinoma in situ (CIS), with no nodal involvement (N0) or distant sites (M0)
	N0	
	M0	
I	T1	The cancer has grown into the layer of connective tissue under the lining layer of the bladder, without reaching the layer of muscle in the bladder wall (T1). No nodal involvement (N0) or distant sites (M0)
	N0	
	M0	
II	T2a or T2b	The cancer involves the inner (T2a) or the outer (T2b) muscle layer of the bladder wall, without completely passing through the muscle to reach the layer of fatty tissue surrounding the bladder. No nodal involvement (N0) or distant sites (M0)
	N0	
	M0	
IIIA	T3a, T3b or T4a	The cancer involves the muscle layer of the bladder, reaching the layer of fatty tissue surrounding the bladder (T3a or T3b). No nodal involvement (N0) or distant sites (M0)
	N0	
	M0	
	OR	
	T1-T4a	The cancer is not growing into the pelvic or abdominal wall (T1-T4a) and it has spread to 1 nearby lymph node in the true pelvis (N1). No distant sites (M0)
	N1	
	M0	
IIIB	T1-T4a	The cancer is not growing into the pelvic or abdominal wall (T1-T4a) and it has spread to 2 or more lymph nodes in the true pelvis (N2) or to lymph nodes along the common iliac arteries (N3). No distant sites (M0)
	N2 or N3	
	M0	
IVA	T4b	The cancer has grown through the bladder wall into the pelvic or abdominal wall (T4b). It might have spread to nearby lymph nodes or not (Any N). No distant sites (M0)
	Any N	
	M0	
	OR	
	Any T	The cancer has spread to distant lymph nodes (M1a)
	Any N	
	M1a	
IVB	Any T	The cancer has spread to 1 or more distant organs (M1b)
	Any N	
	M1b	

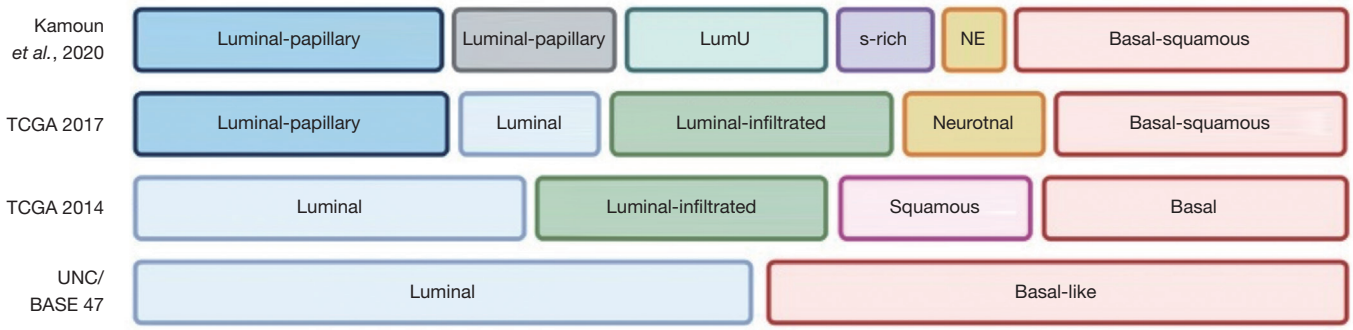

Figure 2 Different subtypes of MIBC according to recent molecular systems. We reported the TCGA 2017, the TCGA 2014 and the UNC/BASE47 systems, with the addition of the recent report by Kamoun and colleagues. More details are reported in the text. LumNS, luminal nonspecified; LumU, luminal unspecified; S-rich, stroma-rich; NE, neuroendocrine-like.

aggressive disease (29). In this regard, several research groups have harnessed the use of NGS technology to reveal the complex and heterogeneous molecular landscape of UC (30).

Nonetheless, the definition of tumor histology, anatomic-based TNM stage and genomic features is not enough. Traditionally, despite platinum-based chemotherapy with cisplatin plus gemcitabine or M-VAC has represented and still represents the standard first-line treatment in metastatic disease, the use of these regimens is widely limited due to related toxicities and patient comorbidities (31). More specifically, around the 50% of metastatic UC are not eligible for cisplatin-based regimen, with cisplatin ineligibility usually defined as follows: ECOG-PS ≥2 and/or creatinine clearance <60 mL/min and/or hearing loss of 25 dB at 2 contiguous frequencies and/or peripheral neuropathy grade ≥2 and/or New York Heart Association class ≥3 heart failure (32). Moreover, age is another important element limiting the use of cisplatin in UC patients (32).

The recent advent of ICIs has changed the front-line setting of cisplatin-ineligible patients, with two trials showing that atezolizumab and pembrolizumab have been suggested to be feasible and effective strategies (33)—we will not discuss the details regarding the trials assessing these and other PD-1 and PD-L1 inhibitors in this setting, that are beyond the scope of this paper. Nonetheless, immunotherapy has its caveats. T-cell activation caused by ICIs can be responsible of immune-related adverse events (irAEs), including skin reactions, thyroid dysfunction, pneumonitis, hepatitis and other toxicities that usually do not occur with conventional cytotoxic chemotherapy (34). More specifically, the incidence of any grade irAEs has been reported to range from 40% to 60% in patients receiving anti-PD-1 and anti-PD-L1 agents, and since irAEs are different from adverse events of systemic chemotherapy, these are frequently underestimated and even not detected (35,36). Lastly, irAEs may led to the necessity of frequent monitoring, to the use of immunosuppressive therapies, and sometimes, to hospitalization and death (37).

Modern oncology has seen a passage from an organ-centric, anatomic-based vision to a deep molecular analysis, moving towards a personalized approach. All things considered, although genomic studies have opened the door of new world, personalized oncology cannot overlook clinical features and underlying comorbidities, two elements which are and remain the mainstay of treatment choices in UC—today as yesterday.

UC: the molecular landscape

The genomic characterization of UC has suggested the presence of different biological subtypes of disease, with a diverse mutational landscape (38). Early reports evidenced the presence of two major groups mimicking the breast cancer classification—luminal and basal, which corresponded to different stages in urothelial differentiation (*Figure 2*) (39). Luminal subgroup was reported to express high levels of low molecular weight keratin 20, PPARG, FGFR3 and uroplakins while the basal subgroup was associated with high levels of EGFR, CD44 and high molecular weight keratins, including keratin 14 and 15. Interestingly, it has been hypothesized that basal cells could present important analogies with triple-negative breast cancer cells, which similarly express high molecular weight keratins and stem cell markers such as CD44 (40). Further

studies have subsequently identified more molecular subtypes of UC, according to the expression of examined genes (*Figure 2*).

The Cancer Genome Atlas (TCGA) project for bladder cancer had the merit to shed light on this complex and underground landscape, with two major studies focusing on DNA, RNA and protein analyses (41,42). The first TCGA study included 131 bladder cancer patients, where the integrated genomic analysis showed high somatic mutation rate (median 5.5/Megabase) and 32 significant gene mutations (41). Moreover, the 69% of bladder cancers presented genomic alterations which in the 44% of cases concerned the receptor tyrosine kinase/MAPK pathway and in the 42% the PI3K/AKT/mTOR, according to the results of this analysis. Interestingly, several alterations in the receptor tyrosine kinase/RAS were identified such as FGFR3 activations, EGFR amplifications, ERBB2 and ERBB3 mutations. This first TCGA report described four cancer subtypes: luminal, luminal infiltrated, basal and squamous (*Figure 2*).

In 2017, the results of the TCGA expanded cohort analysis on 412 chemotherapy-naïve samples of MIBC cases have been published, confirming the high mutation rate which characterizes this malignancy (42); moreover, this report detected 64 significant mutated genes, a higher number compared with the 32 mutations of the 2014 analysis. Interestingly, the 412 MIBCs were split in 5 different expression subtypes according to RNA expression analysis: luminal-papillary (35%), luminal (6%), basal-squamous (35%), luminal-infiltrated (19%) and neuronal (5%) (42), a classification which was also proposed in a view to stratify patients for specific therapeutic options (*Figure 2*). Lastly, the authors defined 4 major groups on the basis of distinct mutational signatures (42).

More recently, an international consensus proposed a MIBC classification on the basis of 1,750 transcriptomic profiles from 18 databases (43). According to this classification, a consensus set of six molecular classes has been defined: luminal papillary (24%), luminal nonspecified (8%), luminal unstable (15%), stroma-rich (15%), basal-squamous (35%) and neuroendocrine-like (3%) (*Figure 2*). Interestingly, these classes differ according to infiltration by immune and stromal cells, oncogenic mechanisms, histological and clinical features, suggesting possible therapeutic implications. For instance, the luminal papillary class showed high rate of FGFR3 mutations and translocations, suggesting that FGFR inhibitors could represent effective treatments in these patients. Conversely, the luminal unstable class presented high rate (76%) of TP53 mutations, the basal-squamous high expression of basal differentiation markers and the neuroendocrine-like subgroup inactivation of TP53 and RB1. According to this report by Kamoun and colleagues, stroma-rich tumors and luminal papillary malignancies had the best prognosis while neuroendocrine-like and basal-squamous tumors presented worse prognosis (43).

Molecular testing and treatment choices

Although the identification of molecular subtypes has the potential to guide disease management and therapeutic choices, prognostic and clinical implications of UC subtypes remain largely unclear (44). Importantly, targeting UCs on the basis of molecular subtypes is a very challenging option, given the not negligible heterogeneities and methodological issues (19). At the same time, an increasing emphasis has been recently placed on potential predictive biomarkers, including FGFR alterations, PD-L1 expression, *DDR* genes, and their relationship with molecular subtypes (45).

The FGFR pathway has been involved in the modulation of several biological processes such as cell survival, proliferation, differentiation and angiogenesis (46). As in the case of other malignancies, the four transmembrane receptor tyrosine kinases FGFR1, FGFR2, FGFR3 and FGFR4 can present molecular alterations in UC and, on the basis of the physiological activity of FGFR, aberrations of this signaling may play an important role as pro-oncogenic drivers (47). The frequency of FGFR3 mutations in MIBC is reported to be less than 25% while activating point mutations are more common in early-stage disease (48). In terms of molecular classes, recent reports have highlighted that the luminal subgroup is associated with lower *CD8+* genes, higher FGFR3 expression and resistance to ICIs (49). Interestingly, these data suggest that patients with FGFR3 aberrations might not benefit from immunotherapy, thus guiding therapeutic choice towards FGFR targeted therapies. An exploratory analysis of IMvigor210 trial reported that luminal I subtype patients had lower PD-L1 immune cell expression and *CD8+* genes expression, thus achieving lower response rates to the anti-PD-L1 atezolizumab (50). Conversely, PD-L1 expression on immunohistochemistry resulted high in the basal subtype, where enriched PD-L1 expression was not related with ORR to the PD-L1 inhibitor, which was in turn significantly higher in luminal cluster II (ORR 34%). Overall, these findings contrast with a similar analysis of the CheckMate275 trial, where patients

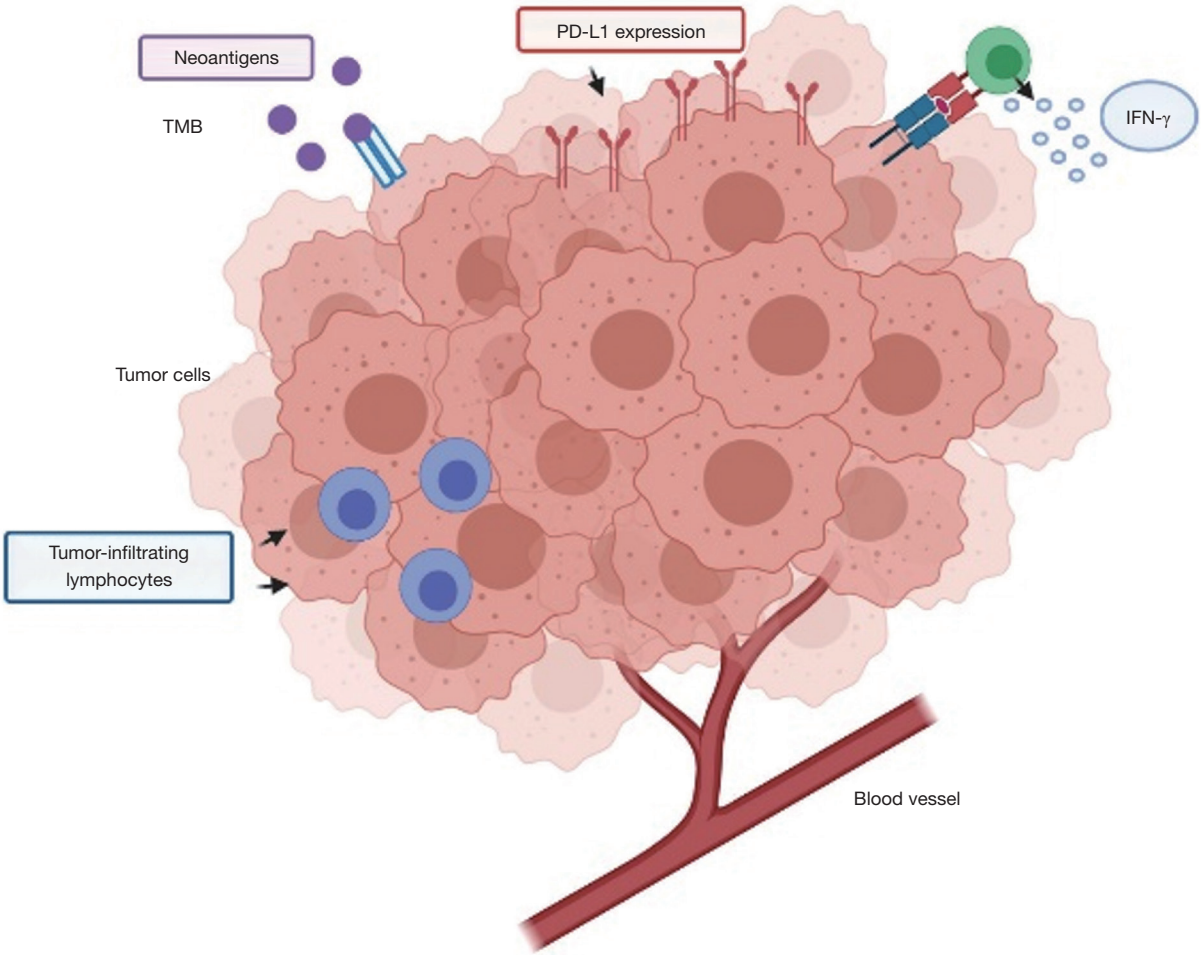

Figure 3 Overview of some of potential biomarkers recently indicated as possible predictors of ICI response in metastatic UC patients. TMB, tumor mutational burden; PD-L1, programmed death-ligand 1; IFN-γ, interferon-gamma.

belonging to the TCGA basal subtype had highest response rate to nivolumab (51). It is worth noting that both studies analyzed biopsies from different specimens for the TCGA analysis, including metastatic lesions, nodal sites of disease and primary tumors. Moreover, the lack of standardization of the TCGA classification in stratifying patients according to molecular subtypes could have represented an important source of bias. Consequently, strong evidence-based conclusions regarding the real impact of TCGA subtyping as a predictive biomarker for ICIs response cannot be drawn so far.

As stated above, although ICIs have shown clinical activity in advanced UC, modifying the therapeutic scenario in this setting, an important percentage of patients does not receive any benefit from immunotherapy due to fast disease progression and lack of response (52). Therefore, biomarkers able to predict response to ICIs would be needed (53). Nonetheless, although some potential associations between biomarkers and responses to immunotherapy have been suggested, these biomarkers have not yet been validated. In terms of predictors of response to ICIs, PD-L1 expression, tumor infiltrating CD8+ lymphocytes and tumor mutational burden (TMB) have been widely studied in several malignancies, including UC (*Figure 3*) (54). With regard to PD-L1 expression, it is worth noting that there is no standardized format to assess PD-L1 with immunohistochemistry (55). Moreover, thresholds to define PD-L1 positivity vary in different trials and methods themselves of evaluation of PD-L1 may be based on immunohistochemistry or tumor cells (56). Overall,

the presence of different assays and scoring systems to define the cut-off positivity for PD-L1 expression is source of confusion, with different trials reporting conflicting results. Probably, the use of a single, standardized method to assess PD-L1 positivity would be the first step to follow, before trying to establish its predictive value in UC patients receiving ICIs.

Another potential predictive biomarker is TMB—commonly defined as the overall number of mutations detected in cancer cells (57). Recent reports have associated increased TMB with more favorable responses to ICIs and, according to results described from the TCGA project, UC presents the third highest mutation rate after melanoma and lung cancer (41). Data from an IMvigor210 subgroup analysis suggested that higher TMB could correlate with clinical benefit, with higher ORR and longer OS in patients receiving atezolizumab (50). Interestingly, median mutation load was 6.4 mut/Megabase in subjects that non responded to atezolizumab compared with 12.4 median mut/Megabase in atezolizumab-responders. Nonetheless, a subsequent reanalysis by whole-exome sequencing did not confirm this association between TMB and response to atezolizumab (58). Lastly, another report from the CheckMate275 trial suggested a correlation between high TMB and better PFS (3.02 versus 1.87 months) and ORR (31.9% versus 17.4%) (51). Prospective data on larger cohorts of patients are still necessary to clarify the role of TMB in UC, a biomarker which undoubtedly needs further validation.

High levels of CD8+ T cells characterize the T cell tumoral inflammation, together with an increased interferon (IFN) and TH1-like chemokine expression (59). When T cells are activated, these are able to proliferate and to differentiate, with subsequent release of pro-inflammatory cytokines (*Figure 3*) (60). These cytokines include IFN-γ, leading to an upregulation of PD-L1 and PD-L2 (61); on the basis of PD-L1 protein expression and the number of tumor-infiltrating lymphocytes (TILs), the tumor microenvironment (TME) is usually defined as non-immunogenic ("cold") or immunogenic ("hot"), and the assessment of TME immunogenicity has been suggested as a useful guide for treatment decision (62). For instance, high levels of IFN-γ and higher density of TILs were associated with increased ORR to atezolizumab in the IMvigor210 trial; similarly, 177 tumor samples correlated to responses to the anti-PD-1 agent nivolumab showed a higher IFN-γ signature in CheckMate275 (50,51). However, these data are still preliminary and further studies are needed since ICIs-responders in these two trials were not only patients with inflamed cytokine signatures.

Other interesting biomarkers are *DDR* genes alterations, which have been associated with a reduced ability to repair DNA damage (63). In fact, the role of DDR genes consists in maintaining genomic stability, repairing DNA damages (64), and in physiological conditions, the mechanism repairs damaged nucleotides without harmful effects; conversely, in presence of DDR genes alterations, repair mechanisms cannot work, resulting in genomic instability (65). More specifically, the DDR pathways are able to recognize DNA damage, to stop cell cycle and to play a fundamental role in DNA repair (66)—where a key element is represented by the Poly (ADP-ribose) Polymerase 1 and 2 (PARP1 and PARP2) genes and whose inhibition surely represents one of the hottest topics in current cancer research (67). Around the 38% of MIBC patients has been reported to present mutations in genes involved in the DDR pathway and previous reports have suggested that *DDR* gene alterations could play a prognostic role in metastatic UC (68); nonetheless, this prognostic role is still to be clarified. In fact, while some reports suggested that tumors with low excision repair cross complementing 1 (ERCC1) mRNA expression could be associated with longer survival (69), other studies have highlighted worse survival in patients with high expression of ERCC1, RAD51 and PAR at immunohistochemistry (70). The prolonged survival of patients harboring *DDR* genes mutations appears intimately linked to the sensitivity of platinum-based chemotherapy, as previously found in other malignancies (e.g., ovarian cancer, breast cancer and pancreatic adenocarcinoma) (71,72). Lastly, higher mutational load and TILs have been identified in patients with DDR gene alterations, thus providing the rationale for the testing of ICIs in this setting (73). Interestingly, a recent retrospective study has detected a statistically significant association between DDR alterations and response to PD-1 and PD-L1 inhibitors in metastatic UC patients receiving nivolumab or atezolizumab (74). Further efforts are warranted in this direction in order to better define if alterations in DDR genes could represent a potential marker of clinical benefit in patients treated with modern immunotherapy.

Conclusions

Despite notable advances in the understanding of molecular features characterizing this disease, metastatic UC remains a difficult to treat malignancy, and therapy is still palliative. A

broad range of recent studies have suggested the presence of UC molecular subtypes, the characteristics and therapeutic implications of whom still need to be clarified. In this changing landscape, more efforts are needed to identify UC patients who are most likely to benefit from medical treatment, whether it is ICIs, targeted therapies or other novel emerging drugs, through a 360-degree evaluation including clinical, anatomic and molecular features.

Acknowledgments

Funding: None.

Footnote

Reporting Checklist: The authors have completed the NARRATIVE REVIEW reporting checklist. Available at http://dx.doi.org/10.21037/tau-20-1109

Provenance and Peer Review: This article was commissioned by the Guest Editors (Rodolfo Montironi, Alessia Cimadamore, Antonio Lopez-Beltran, Marina Scarpelli and Liang Cheng) for the series "Update on Molecular Classification and Individualized Treatments of Genitourinary Tumors" published in *Translational Andrology and Urology*. The article was sent for external peer review organized by the Guest Editor and the editorial office.

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/tau-20-1109). The series "Update on Molecular Classification and Individualized Treatments of Genitourinary Tumors" was commissioned by the editorial office without any funding or sponsorship. AC, MS, LC, ALB and RM served as the unpaid Guest Editors of the series. LC serves as an unpaid editorial board member of *Translational Andrology and Urology* from Dec 2018 to Nov 2022. The authors have no other conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Cite this article as: Rizzo A, Mollica V, Cimadamore A, Santoni M, Scarpelli M, Schiavina R, Cheng L, Lopez-Beltran A, Brunocilla E, Montironi R, Massari F. TNM staging towards a personalized approach in metastatic urothelial carcinoma: what will the future be like?—a narrative review. Transl Androl Urol 2021;10(3):1541-1552. doi: 10.21037/tau-20-1109

