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Precision medicine for urothelial carcinoma: An international perspective

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Abstract

The treatment landscape of urothelial cancers has evolved in the last decade with the approval of chemotherapy, immune checkpoint inhibitors, targeted therapies, and antibody drug conjugates. Although improvements in response and survival have been achieved with these strategies, in some scenarios their benefit is still questionable. Current efforts to identify prognostic and predictive biomarkers are crucial for better patient selection and treatment outcomes. In this paper we will review the most promising biomarkers under investigation, such as molecular classifiers, genomic alterations, programmed cell death ligand 1 expression, tumor mutational burden, circulating tumor DNA, urinary biomarkers among others, for muscle invasive bladder cancer and metastatic urothelial cancers. Deeper understanding of these biomarkers will aid clinical decision-making and help tailor treatment strategies.

Keywords: Antibody drug conjugate; Bladder cancer; Checkpoint inhibitors; Immunotherapy; Precision medicine; Targeted therapy; Urothelial cancer

1. Introduction

Platinum-based chemotherapy was once the only treatment to demonstrate meaningful overall survival (OS) benefit in advanced urothelial cancers (UC) [1]. In the last decade, immune checkpoint inhibitors (ICI) have been approved in the adjuvant setting, [2] as a first-line maintenance therapy [3], as a first-line treatment for chemotherapy-ineligibile or cisplatin-unfit with high-PD-L1 expression [4,5] and as a second-line treatment [6]. Recently, the therapeutic landscape of metastatic UC (mUC) has expanded with the Food and Drug Administration (FDA) approval of erdafitinib for tumors harboring fibroblast growth factor receptor (FGFR) 2- or FGFR3-activating mutation or fusion [7], as well as enfortumab vedotin (EV) [8] and sacituzumab govitecan (SG) [9] in unselected patients after progression to platinum-based chemotherapy and ICI.

Although improvements in response and survival have been achieved with these strategies, their benefit is not universal, therefore identifying prognostic and predictive biomarkers are crucial for better patient selection. In this paper we will review the most promising biomarkers under investigation for bladder cancer in different stages (mainly mUC) aiming to aid clinical decision-making, improve patient outcome and tailor treatment strategies.

2. Materials and methods

We performed an extensive review of medical literature published in peer reviewed journals and meeting abstracts up to June 2023. We selected manuscripts related to precision medicine and urothelial carcinoma and included them in this article, discussing the genomic profile and clinical relevance of genomic alterations and their prognostic and

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predictive relevance in an attempt aid clinical decision-making, improve patient outcome, and tailor treatment strategies.

2.1. Molecular classifications

Genomic alterations have been identified in UC, including PI3K/AKT/mTOR, CDKN2A/CDK4/CCND1, RTK/RAS pathways, ERBB2 (HER2), ERBB3 and FGFR3 as well as mutations in chromatin regulatory genes [10]. Some of those alterations have moved forward the development of new drugs. The better understanding of molecular characteristics of UC has led to disease subclassification. In muscle-invasive bladder cancer (MIBC), the cancer genome atlas (TCGA) research network reported 5 expression subtypes known as luminal-papillary, luminal-infiltrated, luminal, basal-squamous, and neuronal [11]. The luminal-papillary and basal-squamous subtypes correspond to 70% of the cases and the first one is associated to better survival. The luminal subtypes show high expression of uroplakins and urothelial differentiation markers. FGFR3 alterations has a dominant role in luminal-papillary tumours and this subtype is associated with low carcinoma in situ (CIS) expression signature and have non−muscle-invasive papillary bladder cancer (BC) as a precursor. The luminal-infiltrating subtype is associated with chemoresistance, wild-type p53 signature and has increased expression of immune-markers such as programmed cell death 1 (PD-1) or programmed cell death ligand 1 (PD-L1). Otherwise, the basal-squamous subtype has the strongest immune expression signature, and it is characterized by high expression of basal and stem-like markers, enriched TP53 mutations, common in females and has strong expression of CIS signature genes, suggesting CIS lesion as its precursor. Finally, the neuronal subtype has high expression of neuronal differentiation and development genes, and typical neuroendocrine markers as well. Eighty-five percent of those tumours have alterations in genes in the p53/cell-cycle pathway and carry on the worst survival among all subtypes. High mutational load in MIBC is driven mainly by APOBEC-mediated mutagenesis [11]. Translating molecular classification as a strategy for therapy selection in clinical practice is desirable but incipient, however, retrospective analysis have suggested that basal subtype may have a better response to neoadjuvant chemotherapy compared to surgery alone [12]. Additionally, the neuronal subtype seems more responsive to PD-L1 inhibitor atezolizumab with higher survival probability [13].

Kamoun et al. [14] gathered 1,750 MIBC to determine transcriptomic profiles from 16 published datasets and 2 additional cohorts to create a consensus on MIBC molecular classification. Six molecular classes and their respective frequency were identified: luminal papillary (24%), luminal nonspecified (8%), luminal unstable (15%), stroma-rich (15%), basal/squamous (35%), and neuroendocrine-like (3%). Such as TCGA classification, the luminal classes overexpress urothelial differentiation signatures while basal/squamous and neuroendocrine-like overexpress gene signatures associated with basal and neuroendocrine differentiation, respectively. The proposed new class stroma-rich displays intermediate levels of urothelial differentiation and overexpression of smooth muscle, endothelial, fibroblast, and myofibroblast gene signatures. The luminal classes were associated with better outcomes while basal/squamous and neuroendocrine-like with worse prognosis, being the last one the worst among all classes, in alignment with TCGA results [14]. A 4-gene NanoString-based panel related to luminal and basal subtypes has been proposed to classify UC into 3 categories and make it more suitable for use in clinical practice, however, this panel has not yet been validated [15].

Although advances in treatments options for UC have been impressive in the last decade, clinical trials driven by molecular classification are ongoing and the results are awaited.

2.2. DNA damage repair (DDR) pathways

Genomic instability and related DDR pathways are a well-known hallmark of cancer and might drive cancer behavior and response to treatment [16]. Few conventional DDR gene variants have been identified by TCGA, except for *ERCC2* (involved in the nucleotide excision repair pathway) somatic missense mutation, present in 15% of cases [11]. Of note, DNA adducts that are typically repaired by the nucleotide excision repair pathway are consequence of tobacco use, important risk factor for UC, and predicts response to platinum-based treatment, the cornerstone of UC chemotherapy. The association between *ERCC2* mutation and cisplatin sensitivity has been externally validated and patients harboring these mutations have better survival outcomes with neoadjuvant chemotherapy [17].

Beyond *ERCC2*, other DDR genes such as *ATM*, *RB1* and *FANCC* have been described and their variants associated with better outcomes in MIBC patients receiving cisplatin-based neoadjuvant chemotherapy [18]. Data from 2 prospective phase 2 clinical trials, showed that the presence of 1 or more functionally relevant mutations in *ATM*, *RB1,* or *FANCC* was predictive of response to cisplatin-based chemotherapy and associated with longer overall and disease-specific survival when compared with the absence of mutations.

Vidotto et al. evaluated pretreatment tumor transcriptomic profiles in MIBC according to TCGA subtypes to determine subtype specific immune cell abundance, expression of immune regulatory genes and association with DDR gene inactivation. The authors reported *ATM*, *RB1,* and *TP53* were the most frequent inactive genes via biallelic loss, and biallelic mutations in *ATM*, *BRCA1/2*, *PALB2*, *RB1*, and *TP53* were linked to significant increase in immunogenic mutations [19].

The increasing use of immunotherapy in UC treatment should shed light on the relationship between different molecular subtypes, immunomarkers and DDR alterations.

2.3. Urinary biomarkers

Liquid biopsy in urine is a noninvasive strategy and might succeed in bladder cancer as a low morbidity diagnostic or prognostic tool [20,21]. Recently, assays evaluating urine tumoral DNA (utDNA) are being studied for detecting and monitoring for minimal residual disease (MRD) in MIBC and have shown promising results in correlating with pathologic response [22,23].

Urine tumor DNA multidimensional bioinformatic predictor (utLIFE-UC) is a bioinformatic workflow model that uses combined assays for genetic alteration and large copy number variants to identify MRD in MIBC. Initial analysis showed a sensitivity of 100%, specificity of 87.5% and a negative predictive value (NPV) of 100% in the discovery cohort. The validation cohort confirmed the findings, sensitivity of 100%, specificity of 80% and NPV of 100%. The UC MRD score was similar before neoadjuvant therapy. After chemotherapy, prior to surgery the non-pCR group had higher MRD score than the pCR group, supporting that utLIFE-UC MRD detection might be a predictor of pathologic response [24].

Urine Cancer Personalized Profiling by Deep Sequencing (uCAPP-Seq) is a targeted next-generation sequencing (NGS) method for detecting utDNA from urine cell-free DNA (cfDNA) samples. Chauhan et al. showed that the levels of utDNA acquired on the day of curative-intent radical cystectomy correlated with the absence of pCR ($P < 0.001$) with a sensitivity of 81% and specificity of 81%. In addition, utDNA MRD−positive patients had worse progression-free survival (PFS; HR = 7.4; 95% CI: 1.4−38.9; $P = 0.02$) compared to utDNA MRD−negative patients. High concordance (85%) between urine- and tumor-derived mutations was identified, therefore making utDNA a promising method for selecting patients for bladder sparing strategies and potentially for systemic treatments [23]. Although the results are encouraging, these techniques are complex and prospective randomized trials are needed to confirm their clinical utility.

2.4. Circulating tumor (ct) DNA

Advanced UC is a heterogeneous disease [25]. ctDNA supposedly reflects the totality of all tumor sites or at least its predominant clones. The identification of diverse mutations, copy number changes, and chromosomal rearrangements suggests that ctDNA may be useful for detecting mutations associated with response to treatment or characterizing mutational load. The first reports exploring ctDNA in UC employed digital droplet polymerase chain reaction (ddPCR) for highly sensitive detection of distinct UC associated mutations in plasma, specifically for monitoring patients for relapse in the aftermath of local disease intervention [26]. Recently, it has been shown that the presence of ctDNA can identify patients at higher risk of recurrence and progression even before radiological progression [27]. Selecting patients for adjuvant therapy is challenging as there is a need to avoid potential toxicities for those cured by surgery alone. The IMvigor 010 study compared atezolizumab, an anti-PD-L1 inhibitor, to observation after surgical resection. Atezolizumab did not improve disease-free survival (DFS) in unselected patients, but an exploratory analysis showed that patients who had ctDNA clearance with treatment had superior DFS (HR = 0.26, 95% CI: 0.12−0.56, $P = 0.0014$) and OS (HR = 0.59, 95% CI: 0.41−0.86) compared to those who did not have. No difference in clinical outcomes was seen in patients who were ctDNA negative when treated with atezolizumab or observation, suggesting that these patients may not need adjuvant treatment [28].

The ABACUS study, a phase 2 study of neoadjuvant atezolizumab before cystectomy in MIBC, also explored ctDNA as a potential biomarker. The presence of ctDNA correlated with poor outcomes. Eighteen percent of patients treated with atezolizumab had ctDNA clearance, which correlated with pathological response. Patients with unchanged ctDNA levels did not respond to treatment [29]. Together, these findings suggest that ctDNA can be a marker for MRD and will probably change our understanding and clinical trial designs in the perioperative setting.

A cohort exploring the use of ctDNA in upper tract UC (UTUC) was 100% specific for no nonmuscle invasive disease pTis, pTa, pT1, or pN0, suggesting that ctDNA analysis is a feasible nonsurgical approach to predict high-risk UTUC and may select patients for NAC [30].

In some studies, the clinical sensitivity for ctDNA to detect subsequent radiological relapse was approximately 60% and it holds a promising role in detecting early response and progression prompting change in therapy. Challenges such as low levels of ctDNA, nonshedding tumors, timing of testing, and indolent disease limit the use of ctDNA in clinical practice. Prospective trials are needed to confirm the role of ctDNA before it can be routinely adopted in clinical practice [31].

2.5. Programmed cell death ligand 1 (PD-L1)

PD-L1 expression has been widely explored as a potential biomarker for immunotherapy response, but in UC it has had limited applicability. The KEYNOTE-045 trial randomized patients who progressed on first line platinum-based chemotherapy to chemotherapy or pembrolizumab and demonstrated that pembrolizumab conferred an OS benefit (10.3 vs 7.4 months, HR = 0.73) and increased response rate (21.1% vs. 11.4%) compared to chemotherapy. Although the benefit was higher in those with a combined positive score (CPS) greater than 10, responses were also seen in patients with CPS < 10, rendering a limited use for PD-L1 testing in this scenario [6]. Currently, cisplatin-

ineligible patients should only be considered for first-line treatment with ICI (pembrolizumab [4] or atezolizumab [5]) if a high PD-L1 expression (HR = 0.68, 95% CI: 0.43 −1.08) is detected as phase 3 trials suggested shorter OS in patients with low/no PD-L1 expression (HR = 1.07, 95% C: I 0.86−1.33) when treated with ICI monotherapy (13.5 months) when compared to platinum-based chemotherapy (12.9 months) [32,33].

Since 2020, the standard first-line treatment for mUC is platinum-based chemotherapy followed by maintenance avelumab for patients without disease progression. This strategy rendered 9.2 month increase in OS for patients treated with maintenance avelumab, irrespective of PD-L1 expression [3]. Thus, in UC PD-L1 expression has limited use for predicting benefit to ICI.

2.6. TMB and mutational signatures

BC is a highly mutated tumor, and a high mutational burden has been linked to ICI response. In 2020, the results of a phase 2 trial led to tissue-agnostic approval of pembrolizumab due to increased objective response rate (ORR) in patients with high TMB. [34] Across different tumor types, a positive correlation between high TMB (RR for UC 5% vs. 30%) and response to ICI has been shown [35]. Although, Keynote-158 trial defined a high TMB as equal or greater than 10 mutations/megabase, there is evidence to suggest that a different cut-off should be used for each cancer type [36]. In addition to the mutational burden, mutational signatures, such as APOBEC signature, have been shown to be the most important predictor to ICI response as shown in a recently published meta-analysis [37]. Prospective validation of these biomarkers is needed before it can be routinely used for treatment selection in clinical practice.

2.7. FGFR alterations

FGFR gene alterations are found in different tumor types, and it is investigated as an agnostic biomarker with promising results (ORR 29.5%) [38]. For UC this is already a stablished predictive biomarker and erdafinitib, a pan *FGFR* inhibitor, has been FDA-approved since 2019 based on the results of the BCL2001 trial which showed an ORR 40%, with 3% complete response (CR) rate, a median (m) PFS of 5.5 months and a mOS of 13.8 months [7]. Recently, the results of THOR trial, a randomized phase 3 trial, showed that erdafitinib is superior to standard chemotherapy in *FGFR*-altered tumors after progression to platinum-based chemotherapy and ICI with a decreased risk of death of 36%, mOS of 12 months, mPFS of 5.6 months and an ORR of 46% [39].

A phase 2 trial comparing rogaratinib with chemotherapy in patients with *FGFR* mRNA-positive (overexpression of *FGFR1* or *FGFR3* mRNA) tumors did not show significant difference in ORR, OS and PFS between the 2 arms. An exploratory analysis showed an improved ORR with rogaratinib in FGFR mRNA-positive patients with FGFR genetic DNA alterations therefore suggesting that the method used to identify *FGFR* alterations may impact on patient selection for therapy [40]. Other *FGFR* inhibitors are under development (pemigatinib, derazantinib, rogaratinib) as well as new combinations trials are awaited (NCT05564416, NCT03473756, NCT04963153)

Patients should be selected for therapy based on an FDA-approved companion diagnostic for erdafitinib, the QIAGEN therascreen® *FGFR* RGQ RT-PCR Kit [7]. Although tissue testing is the gold standard, it has been shown in longitudinal sequencing studies that *FGFR* status can be variable overtime, therefore highlighting the need for other tests such as ctDNA and urine biomarkers to facilitate repeated testing, accurate screening and determining systemic therapy [41].

Data suggests that UC with alterations in *FGFR* genes are less responsive to ICI [7]. The immune microenvironment of these tumors could be altered by suppression of interferon signaling pathway. In THOR trial it has been shown that 90% of the tumors had CPS < 10 and other series have also shown that *FGFR*-altered tumors have higher frequencies of biomarkers predicting ICI resistance, such as: low TMB and PD-L1 expression, higher frequencies of genomic alterations in *MDM2*, *CDKN2A*/B, and *MTAP* loss, eliciting further investigation on assessment of response to ICI of *FGFR* altered tumors [42]. The ongoing cohort 2 of the THOR trial that is comparing erdafitinib to pembrolizumab in *FGFR*-altered tumors after the progression to platinum-based chemotherapy and results are awaited.

The NORSE trial compared the combination of cetrelimab (anti-PD-1) and erdafitinib to erdafitinib as first-line treatment for cisplatin-ineligible patients, the primary endpoint was ORR. The ORR for the combination vs. erdafitinib was 54.5% vs. 44.2%, with 13.6% vs. 2.3% CR. The 12-month OS was 68% vs. 56% [43]. These results demonstrated the clinical activity of the combination, erdafitinib, and cetrelimab, but it specially highlights and confirms the activity of *FGFR* inhibitor in *FGFR*-altered tumors.

2.8. Biomarkers for antibody drug conjugate (ADC)

ADCs comprise a therapeutic class of agents with well-defined targets in cancer cells, offering a novel approach in the management of UC. The FDA-approved ADCs, EV, and SG, are designed to specifically target Nectin-4 and Trop-2 antigens, respectively, which are consistently overexpressed in UC across diverse disease stages [44]. Consequently, clinical trials designed to assess the safety and efficacy of these ADCs have explored its use in unselected patients, emphasizing its efficacy and capacity to bypass initial tumor biomarker testing [45,46]. However, human epidermal receptor 2 (HER2), a target of significant interest given its therapeutic success in other malignancies, also presents a viable alternative in UC with potential role in disease progression and prognosis [47].

2.9. NECTIN-4 / other biomarkers being studied for EV

Nectin-4 is a calcium-independent immunoglobulin-like cell-cell adhesion molecule of the nectins family, encoded by the PVRL4 gene, also known as poliovirus receptor-related protein 4, highly expressed in BC. From preclinical studies, the use of EV, a fusion between a fully human antibody targeting nectin-4 and the potent microtubule-disrupting agent monomethyl auristatin E (MMAE), demonstrated inhibition of in vitro growth of cell lines that express cell surface nectin-4. In this preclinical model, the positivity rate of nectin-4 expression in transitional BC tumor specimens ($N = 467$) was 83% [48]. In the phase 1 clinical trial exploring EV for the treatment of refractory UC, the nectin-4 expression, as determined by immunohistochemical H-score was high for the majority of the samples (median H-score 290), and only 5 out of 152 samples showed a H-score of < 150, which led to the conclusion of the authors in that paper that prescreening of nectin-4 expression was not necessary for administration of EV in UC [49]. Although not formally statistically tested, the distribution of nectin-4 expression was similar between responders and nonresponders (median H-score 270 [IQR 230−295] and 280 [IQR 233−330], respectively) in the analysis of cohort 2 of the phase 2 EV-201 clinical trial [50]. Those findings resulted in the inclusion of unselected patients in the pivotal phase 3 EV-301 trial, which led to the regulatory approval of EV for previously treated advanced UC [46]. However, in a retrospective analysis of 137 specimens of patients with UC treated with EV, nectin-4 expression significantly decreased during metastatic spread of disease ($P < 0.001$), and low or absent membranous nectin-4 expression was predictive of resistance to EV treatment, and absent or weak tumoral nectin-4 expression demonstrated an increased risk of progression during treatment when compared to moderate or strong expression on multivariable-adjusted analysis (HR = 4.26; 95% CI: 1.55−11.70; $P = 0.005$) [51]. In a recent retrospective analysis of the UNITE study, NGS data were used to assess the TMB, PD-L1 status, somatic alterations, and mutation in DDR genes. Patients with alterations in *ERBB2* (67% vs. 44%; $P = 0.05$) and TSC1 (68% vs. 25%; $P = 0.04$) demonstrated higher response rates when compared to patients with wild-type genes. The assessment of PFS showed shorter outcomes in patients with mutations in *CDKN2A* (4.6 vs. 6.0 months; $P = 0.002$), *CDKN2B* (4.4 vs. 6.0 months; $P < 0.01$) and *MTAP* (4.6 vs. 6.0 months; $P = 0.005$), while patients with high TMB experienced prolonged OS (13.6 vs. 8.3 months; $P = 0.02$) [52].

2.10. Trop-2

The transmembrane glycoprotein Trop2, which is encoded by the Tacstd2 gene, functions as an intracellular calcium signal transducer that is differentially expressed in numerous cancers with stem cell-like properties for the regulation of growth, transformation, regeneration, and proliferation. Trop2 overexpression is linked to the activation of the *ERK/MAPK* pathway, resulting in downstream modifications in the cancer cell cycle and the promotion of cell growth. Trop2 is overexpressed in a range of human carcinomas, such as breast, cervix, colorectal, esophagus, lung, non-Hodgkin's lymphoma, chronic lymphocytic lymphoma, Burkitt lymphoma, oral squamous cell, ovarian, pancreatic, prostate, stomach, thyroid, uterine, and urinary bladder, and has been linked to inferior survival outcomes, increased aggressiveness, and metastasis capacity [53]. SG is an ADC composed of an anti-Trop-2 humanized monoclonal antibody coupled to SN-38, an active metabolite of irinotecan, a topoisomerase I inhibitor. Initial results of the phase 1/2 clinical trial assessing the activity of SG in patients with advanced epithelial cancers who had received at least 1 prior therapy for metastatic disease showed encouraging clinical activity across various solid tumors, including patients with mUC, with an ORR of 31% (14/45) [54]. In the subsequent phase 2 TROPHY-U-01 multicohort trial, cohort 1 included 113 patients with locally advancer or unresectable or mUC who progressed after prior platinum-based chemotherapy and ICI. The ORR in that population was 27.4% (95% CI: 19.5−36.6), including 5.3% confirmed CR. Of interest, in patients previously treated with EV ($N = 10$), the anti-Trop-2 ADC demonstrated similar efficacy, with 30% ORR (95% CI: 6.7−65.3) [9]. Those encouraging results led to the accelerated regulatory approval by the FDA, while the confirmatory phase 3 TROPICS-04 trial in currently ongoing for the comparison between SG and chemotherapy (docetaxel, paclitaxel or vinflunine) in patients with advanced UC who progressed after prior platinum-based chemotherapy and ICI [45].

2.11. ERBB2

The human epidermal growth factor receptor 2 (*HER2*), a member of the *EGFR* family of receptor tyrosine kinases, is frequently overexpressed in numerous malignancies, contributing significantly to cellular proliferation and tumorigenesis [47,55]. In the context of BC, up to 12.4% of specimens have demonstrated *HER2* overexpression (IHC 3 +), while missense single-nucleotide variants in *ERBB2* have been identified in 11% of MIBC. In addition, 19.2% of patients present with *ERBB2* amplifications. Despite the exploration of several therapeutic strategies for HER2-altered advanced urothelial cancer, including trastuzumab, pertuzumab, lapatinib, afatinib, neratinib, and trastuzumab emtansine, no unequivocal benefit has been demonstrated across these drug classes [47]. However, the advent of novel and more efficacious ADCs has renewed interest in this strategy, particularly with the introduction of trastuzumab deruxtecan and disatamab vedotin, which may be administered in combination with ICI [56−59]. Furthermore, these novel agents hold promising potential for treating patients with low HER2 expression (immunohistochemistry 1+ or

2+ with in situ hybridization negative), representing up to 17.5% of cases [60] with ORR of 35% [61]. This development broadens the patient pool eligible for this treatment strategy, marking a significant advance in personalized oncological care.

2.12. J. future directions

In the search for different targets for the development of personalized treatment of UC, the science field has explored some oncogenic pathways often disturbed during carcinogenesis processes of this tumors. However, results of these studies have not demonstrated any relevant findings so far.

2.13. PI3K/AKT/mTOR, MAPK pathway

The phosphatidylinositol 3-kinase (*PI3K*) signaling pathway is pivotal in regulating cellular metabolism, migration, proliferation, and survival. Aberrant activation of this pathway, often due to mutations, is observed in nearly all cancer types. In BC, mutations predicted to activate the *PI3K* pathway are present in 50% to 70% of tumors, and phosphorylation status assessments confirm pathway activation across all tumor grades and stages. This suggests a potential benefit of *PI3K*-targeted therapy for these tumors [62].

Despite the prevalence of *PIK3CA* mutations, the most common *PI3K* pathway mutations in BC, including in 12% to 20% of muscle-invasive tumors, *PI3K* inhibition as a therapeutic approach in UC has been relatively unexplored [10]. However, preclinical studies and early-phase clinical trials have demonstrated sensitivity to *PI3K* inhibitors in various cancers, including breast, ovarian, endometrial, lung, and multiple myeloma [63]. Emerging preclinical data also underscores the potential synergistic effect of combining *PI3K* inhibition with immunotherapy for UC harboring *PI3K/AKT/mTOR* pathway genomic alterations [64]. Additionally, UC with *ARID1A* mutations, accounting for up to 20% of cases, appear sensitive to *PI3K* and *EZH2* inhibition, based on xenograft models [65]. These findings indicate potential new directions for personalized treatment strategies in UC.

2.14. Other TKI and VEGF inhibitors

Tyrosine kinase inhibitors (TKIs) and vascular endothelial growth factor (VEGF) inhibitors represent an exciting frontier in the treatment of UC. Notably, multitargeted TKIs such as sunitinib, which inhibits VEGF, PDGF, and c-kit receptors, and pazopanib, a VEGF receptor inhibitor, have demonstrated promising antitumor activity in preclinical and early clinical trials, which was not confirmed in latter studies [66,67]. Similarly, VEGF inhibitors, such as bevacizumab, have shown potential in limiting tumor angiogenesis, a critical process in tumor growth and metastasis, but addition of this antibody to chemotherapy failed to improve survival outcomes in randomized trial [68]. The understanding of the predictive biomarkers for response to these inhibitors could further refine personalized treatment approaches to establish their place in the therapeutic armamentarium against UC.

2.15. International perspective

The world is witnessing important advances in the treatment of urothelial cancer, but there is a worldwide heterogeneity in adoption and availability of these therapies in clinical practice, mainly due to differences in approval of regulatory agencies and financial costs. Non-United States (US) and non-European populations are underrepresented in clinical trials and there is limited data on epidemiological and molecular characterization of urothelial cancers in these regions. In Latin America, the study LACOG 1518 attempted to establish the *FGFR* alterations prevalence, clinicopathological characteristics and outcomes of patients with advanced UC. *FGFR* alterations were identified in 14.8% of patients, a similar percentage when compared to other regions of the world, but only 1.4% of patients received *FGFR* inhibitors as a treatment option, suggesting limited access to *FGFR* inhibitors in Latin America. [69] In Asia, only 5 of the 9 ICI marketed in the US are approved and there is a limited number of indications and tumor types for which ICI is approved when compared to the US. [70] In China for UC there are only 2 approved indications for ICI compared to 11 in the US. [70] This data highlights the differences and limitations of drug access and implementation of innovative technology in clinical practice in developing nations.

In addition to distinct patterns of drug access and approval, we should consider potential differences in molecular and genomic alterations found in populations with distinct ethnic backgrounds. For instance, Wu et al reported that 20.1% of Chinese patients with UTUC had pathogenic germline mutations. Overall, a similar frequency in alterations in DDR genes was seen between the Chinese and Western populations, The mutation frequency for *BRCA* 2 (3.2% vs. 0.88%) and for MMR genes (4.2% vs. 8.8%) differed between the Chinese and Western populations, respectively (Figure 1).

3. Conclusions

The treatment landscape of urothelial cancers is rapidly changing with the approval of new therapies. Improving outcomes and establishing the optimum treatment sequencing and combinations should be a priority. The identification of predictive biomarkers and molecular subtypes can potentially aid treatment selection, but at this point only *FGFR* alterations and PD-L1 expression are routinely used in clinical practice for therapy selection. Promising data has emerged with *HER2*-low and deruxtecan, but biomarkers with other ADCs remain unexplored. Although a signal is observed with TMB, benefits are also evident in cases with

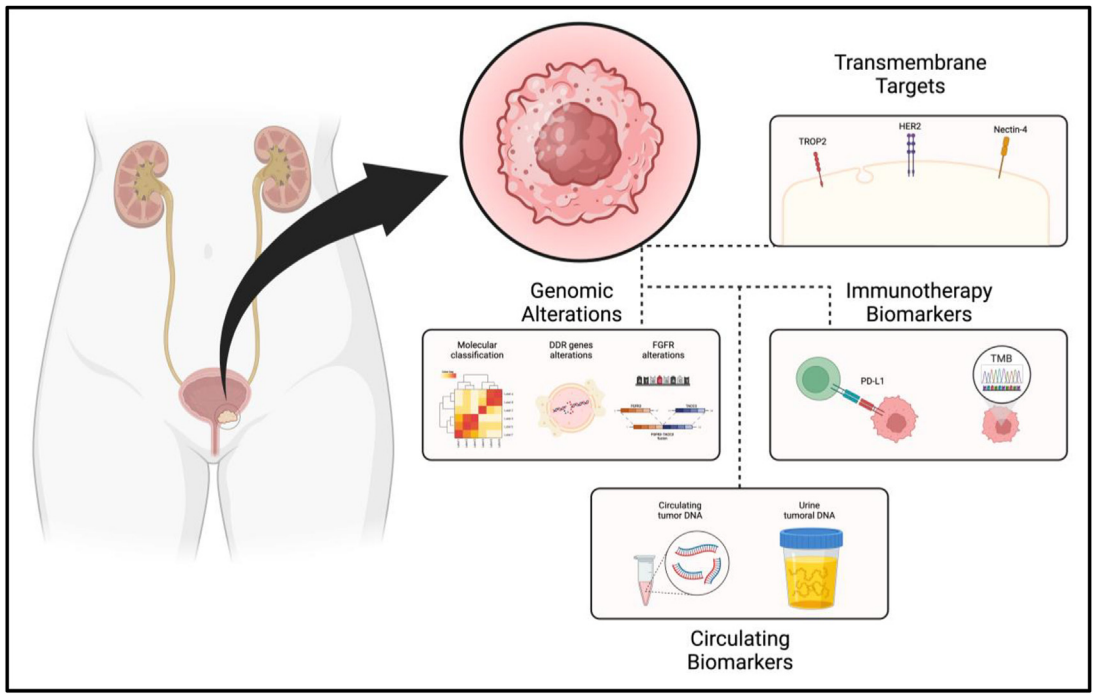

Figure 1. Critical targets explored in precision medicine for urothelial carcinoma.

Abbreviations: DDR = DNA damage repair; FGFR = fibroblast growth factor receptor; HER2 = human epidermal growth factor receptor 2; Nectin-4 = Nectin cell adhesion molecule-4; PD-L1 = programmed cell death ligand 1; TMB = tumor mutational burden; TROP2 = trophoblast cell surface antigen 2.

low TMB and low PDL1. ctDNA is also promising for MRD and adjuvant therapy selection. Prioritizing non-US and non-European populations in urothelial cancer biomarker-driven trials is crucial to broaden the future impact of new technologies worldwide.

Declaration of competing interest

All authors of this manuscript have directly participated in planning, execution, and/or analysis of this study. The contents of this manuscript have not been copyrighted or published previously. The contents of this manuscript are not now under consideration for publication elsewhere. The contents of this manuscript will not be copyrighted, submitted, or published elsewhere while acceptance by Urologic Oncology: Seminars and Original Investigations is under consideration. There are no directly related manuscripts or abstracts, published or unpublished, by any authors of this manuscript. No financial support or incentive has been provided for this manuscript.

I am one author signing on behalf of all coauthors of this manuscript, and attesting to the above.

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