Precision Medicine in Bladder Cancer

Subjects: Pathology

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Bladder cancer (BC) is characterized by significant histopathologic and molecular heterogeneity. The discovery of molecular pathways and knowledge of cellular mechanisms have grown exponentially and may allow for better disease classification, prognostication, and development of novel and more efficacious noninvasive detection and surveillance strategies, as well as selection of therapeutic targets, which can be used in BC, particularly in a neoadjuvant or adjuvant setting.

precision medicine bladder cancer molecular biomarkers histologic subtypes/variants

1. Introduction

Bladder cancer (BC) is the tenth most commonly diagnosed cancer with an age-standardized incidence rate (per 100,000 person/years) of 9.5 in men and 2.4 in women; globally, the age-standardized mortality rate (per 100,000 person/years) is 3.3 for men and 0.86 for women [1][2][3][4]. It is a major cause of cancer-related morbidity and mortality. According to the GLOBOCAN 2020 data, 573,278 new cases and 212,536 deaths of BC are added each year [5]. It typically affects patients in the fifth to seventh decade with a fourfold higher incidence among males [5]. BC exhibits significant morphological and molecular heterogeneity. However, despite its highly characterized molecular signature and high rate of potentially actionable genomic alterations, there has been limited success in various promising biomarker therapies [6].

2. Biomarker

A biomarker is a measured substance or variable whose presence is indicative of or a surrogate for a disease outcome. The potential roles of a biomarker in MIBC include (1) identifying high-risk patients, e.g., patients planned for radical cystectomy ± neo adjuvant therapy, (2) predicting resistance to chemotherapy/immunotherapy, and (3) identifying pathways involved in targeted therapy. A biomarker may be prognostic (in that it provides information about the patient's overall cancer outcome, regardless of the therapy), predictive (in that it provides information about the effect of a therapeutic intervention and, hence, can be a target), or both. An ideal biomarker is one which is reproducible, accurate, validated in multiple datasets, and most importantly, easy to use.

3. Biomarkers for Advanced Urothelial Cancers

3.1. Biomarkers for Response to Chemotherapy (Table 1)

Cisplatin-based chemotherapy (MVAC: methotrexate, vinblastine, doxorubicin, and cisplatin; GC: gemcitabine, cisplatin/carboplatin) is the treatment of choice in patients with metastatic UC of the bladder. The overall response rates (ORRs) range from 60% to 70%, overall survival (OS) ranges from 14 to 15 months, and 5year OS ranges from 13% to 15% [7]. In patients who relapse after platinum-based chemotherapy, ORRs range from 5% to 29% with a median OS of 6.9 months (based on clinical trials of second-line chemotherapy with paclitaxel and vinflunine) [8]. In the neoadjuvant and adjuvant settings in UC, similar regimens are used to those in the metastatic setting. Most data on chemotherapy biomarkers are available for MIBC, since a pathological complete response (pCR) to platinum-based chemotherapy is prognostic in this setting.

Table 1. Biomarkers for response to chemotherapy.

Molecular Target	Study [Ref.]	Results	Comments
DDR Genes			
NER pathway *ERCC1* expression levels	**Bellmunt et al.** [9]	Reduced levels of *ERCC 1* mRNA expression were associated with improved survival to cisplatin-based chemotherapy in mUC.	DDR genes are not validated biomarkers for response to chemotherapy (not routinely used in clinical practice). Clinical trials are evaluating the role of PARP inhibitors in DDR gene mutated UC [10].
	Urun et al. [11]	*ERCC1* positivity was associated with poor survival in mUC treated with cisplatin-based chemotherapy.	
***ERCC2* mutations**	**Van Allen et al.** [12], **Liu et al.** [13]	ERCC2 mutations were associated with pCR and improved OS to neoadjuvant cisplatin-based chemotherapy in MIBC.	
	Kim et al. [14]	*ERCC2*-associated mutation signature single-base substitution 5 (SBS5) was associated with improved responses in mUC.	
HRR pathway *BRCA* mutations	**Taber et al.** [15]	*BRCA2* mutations were associated with SBS5 signature and responses to platinum-based chemotherapy in MIBC	
***RAD51* mutations**	**Mullane et al.** [16]	High nuclear staining for *RAD51*was associated with poor outcome (worse OS) for mUC patients treated with cisplatin-based chemotherapy.	
Other DDR genes *ATM/RB1/FANCC*	**Plimack et al.** [17]	*ATM/RB1/FANCC* mutations were associated with improved	

Molecular Target	Study [Ref.]	Results	Comments
DDR Genes			
mutations		pathologic responses and survival in MIBC treated with neoadjuvant platinum-based chemotherapy.	
HER2/ERBB2 alterations	Groenendijk et al. [18]	*HER2* missense mutations (not amplifications) were associated with response to neoadjuvant chemotherapy with platinum in MIBC.	
Molecular subtypes of bladder cancer	Kamoun et al. [19]	None of the subtypes were found to be associated with neoadjuvant chemotherapy response.	
	Choi et al. [20]	The p53-like subtype was chemo resistant.	
	McConkey et al. [21]	The basal subtype was associated with the most optimal OS in the trial of neoadjuvant chemotherapy MVAC with bevacizumab.	
	Taber et al. [15]	The basal/squamous consensus subtype was associated with reduced neoadjuvant chemotherapy response.	

3.2. Cisplatin Eligibility

Cisplatin eligibility is defined as an Eastern Cooperative Oncology Group performance status > 2, neuropathy/hearing loss grade ≥ 2, creatinine clearance < 60 mL/min, and New York Heart Association heart failure grade ≥ 3 [22][23]. Treatment with cisplatin may be prognostic in metastatic UC (mUC). Patients eligible for cisplatin and treated with cisplatin-based chemotherapy had an improved OS as compared to eligible patients not treated with cisplatin [24]. Thus, treatment with cisplatin in eligible patients (cisplatin utilization) rather than cisplatin eligibility may be a clinical biomarker for improved OS and is of paramount importance, as, even in eligible patients, around one in four is not exposed to this chemotherapy. Cisplatin-ineligible patients treated with carboplatin have a better outcome as compared to non-platinum-based chemotherapy. Lastly, the receipt of any chemotherapy leads to improved survival in comparison to no receipt of chemotherapy [24]. These data are primarily based on retrospective analysis and, hence, should be interpreted with caution.

3.3. DNA Damage Repair Genes (DDR Genes)

Platinum-based chemotherapy leads to DNA damage through the formation of adducts and ultimately apoptosis. In a normal cell, in response to DNA damage, the DDR pathway is activated to repair the damage. This pathway comprises the nucleotide excision repair (NER) for single-stranded DNA damage, the homologous recombination repair (HRR) for double stranded DNA damage, and the Fanconi anemia pathway. Most importantly, mutations in the DDR genes lead to increased susceptibility of cancer cells to platinum-based therapy [25].

3.4. NER Pathway

The excision repair cross-complementation group 1 (ERCC1) protein heterodimerizes with ERCC4 to form an endonuclease complex. This participates in the excision of the damaged DNA. Lower *ERCC1* levels (mRNA expression or IHC) are correlated with cisplatin sensitivity in MIBC and mUC, with improved outcomes in these patients [9]. Conversely, ERCC1 overexpression is associated with worse OS in mUC [11]. ERCC 2 mutations are associated with pCR and improved OS to neoadjuvant cisplatin-based chemotherapy in MIBC [12][13]. *ERCC2*-associated mutation signature single-base substitution 5 (SBS5) is associated with improved responses in mUC [14]. In another study on neoadjuvant GC chemotherapy in MIBC, alterations within a panel of 29 DDR genes were correlated with chemotherapy response. Deleterious DDR gene alterations include nonsense, frameshift, and splice site alterations or*ERCC2*missense mutations. The positive predictive value of a somatic deleterious DDR gene alteration for response was 89%, and the 2 year relapse-free survival was higher in patients whose tumors had a deleterious DDR gene alteration [26].

3.5. HRR Pathway

HRR is a DNA repair mechanism, involved in the repair of double-stranded breaks and interstrand crosslinks. The undamaged homologous chromosome serves as a template for the repair of the damaged strand. *BRCA1* and *BRCA2* are prototypes for HRR genes, known for their roles as cancer predisposition genes and as predictive biomarkers for sensitivity to poly ADP-ribose polymerase (PARP) inhibitors and platinum-based chemotherapy [27]. Somatic *BRCA1/2* alterations were present in 19% of MIBC samples in TCGA, and germline *BRCA1/2* variants were observed in 2–4% of UC patients [28][29][30]. In a recent multi-omics analysis of 300 patients with MIBC or mUC, *BRCA2* mutations were associated with the SBS5 mutation signature and with chemotherapy response [27].

3.6. 3ATM Serine/Threonine Kinase, Retinoblastoma Transcriptional Corepressor 1, or FA Complementation Group C (ATM/RB1/FANCC) Mutations

While *ATM* and *RB1* are cell-cycle regulators in response to DNA damage, *FANCC* is critical in interstrand crosslink repair [27]. Among other DDR genes, *ATM/RB1* mutations are considered as biomarkers of poor prognosis in unselected UC patients and may correlate with higher mutational load. *ATM/RB1/FANCC* mutations are associated with $p <$ T2 response to NAC and improved OS in MIBC [17][31]. An association was also observed between high mutation burden and deleterious DDR genes. However, DDR alterations have no prognostic impact in the absence of NAC [18].

3.7. Other Alterations

ERBB2 (erb-b2 receptor tyrosine kinase 2) missense mutations were associated with response to platinum-based neoadjuvant therapy in MIBC [12][18]. Some other studies found no benefit of *HER2* alterations and response to chemotherapy [17]. Therefore, further analysis is required to determine the above association.

3.8. Molecular Classifications

A gene expression profile (GEP) for a tumor is derived from the extraction and quantification of tumor RNA. GEP may open up avenues for response assessment for antitumoral therapy at the molecular level. On the basis of similarities in the GEP, clustering algorithms may be used to group tumors into molecular subtypes [27]. Accordingly, six consensus molecular subtypes for MIBC have been suggested: basal/squamous, luminal papillary, luminal unstable, luminal non specified, stroma-rich, and neuroendocrine-like; according to their similarity to basal and luminal breast cancer subtypes [28]. The utility of molecular subtypes as predictive biomarkers of chemotherapy response is unclear, and studies have produced conflicting results. The p53-like subtype included under the stroma-rich consensus subtype has been reported as chemoresistant in UC.The basal/squamous consensus subtype has been suggested to be chemoresistant in others. The basal-type tumors were shown to be the most chemosensitive in some studies [20][21]. Lastly, none of the consensus subtypes were found to associate with NAC response in the study by Kamoun et al. [19]. Co-expression extrapolation (COXEN) is a gene expression-based predictive biomarker analysis that identifies gene expression signatures in cancer cell lines associated with in vitro chemotherapy sensitivity and extrapolates those signatures to predict chemosensitivity in vivo. This model has not been shown to predict response to platinum-based chemotherapy in UC [32][33].

4. Biomarkers for Response to Immunotherapy in UC (Table 2)

In the front-line setting, ICI monotherapy has demonstrated activity in cisplatin-ineligible patients [12][22]. The choice between ICIs and carboplatin chemotherapy in this setting is not straightforward. For patients who are platinum-ineligible, ICI monotherapy is a reasonable option. However, for those who are eligible for carboplatin-based chemotherapy, a maintenance ICI approach (per JAVELIN Bladder 100) may be favored over upfront ICI monotherapy, given its proven OS benefit, as described below [34]. PD-L1 expression is used as a biomarker among cisplatin-ineligible patients to choose between ICI monotherapy and carboplatin-based chemotherapy. For patients with PD-L1^{low} tumors, upfront ICI monotherapy may be deleterious according to data on early mortality in the IMvigor130 and KEYNOTE-361 trials [35][36]. Cisplatin-ineligible patients with PD-L1-positive tumors may be considered for upfront ICI or chemotherapy followed by maintenance ICI; these options have not been directly compared in clinical trials. Currently, the strongest evidence for ICI benefit in mUC is in the post-platinum-based chemotherapy setting. Two randomized trials—KEYNOTE-045 and JAVELIN Bladder 100—have demonstrated OS benefits for single-agent ICIs as either second-line or maintenance therapy after platinum chemotherapy [34][37]. Both trials met their primary endpoint of OS in biomarker-unselected, all-comer population. Notably, the use of ICI at progression was permitted in the control arm of JAVELIN Bladder 100, and around one-third of the patients received it [38]. On the basis of these data, a strategy using maintenance ICI is preferred after platinum-based

chemotherapy rather than ICI at progression. This is also the preferred approach per National Comprehensive Cancer Network (NCCN) guidelines, although there exists a risk of over-treatment in some patients.

Table 2. Biomarkers for response to immunotherapy.

Biomarker	Study [Ref.]	Results	Comments
PD-L1	IMvigor 130 [35]; Keynote 361 [36]	Cisplatin-ineligible mUC with PD-L1^{low}did not benefit from ICI monotherapy as compared to chemotherapy.	PD-L1 is a biomarker in cisplatin-ineligible patients to guide the choice of upfront ICI monotherapy vs. carboplatin chemotherapy. In this population, therapeutic choices are carboplatin-based chemotherapy followed by maintenance immunotherapy.
		Cisplatin-ineligible patients with PD-L1-positive tumors benefited from ICI monotherapy.	In this population, options are upfront ICI or chemotherapy followed by maintenance ICI; these options have not been directly compared in clinical trials.
	Rui et al. [39], Litchfield et al. [40]	Meta-analyses of prospective trials showed that, overall, PD-L1 expression was associated with radiographic response to ICIs in mUC patients.	PD-L1 expression is the only ICI biomarker that has been incorporated into mUC regulatory approvals and treatment guidelines. PD-L1 as a biomarker is dynamic in both space and time.
Tumor mutational burden	Galsky et al. [41]	Exploratory analyses of prospective trials in mUC suggested that the combination of TMB and PD-L1 could more effectively distinguish ICI responders and non-responders than either biomarker alone.	Challenges in implementing TMB as a biomarker include selecting an optimal cutoff and harmonizing assays.

Biomarker	Study [Ref.]	Results	Comments
	Litchfield et al. [40]	Clonal TMB and the APOBEC signature were among the most important features associated with response in a multivariable model predicting ICI response in bladder cancer.	
Somatic alterations			
TRAF2	Litchfield et al. [40]	Loss of TRAF2 was associated with ICI response.	
CCND1 amplification	Litchfield et al. [40]	CCND1 amplification was associated with ICI resistance.	DDR genes alone are probably not predictive of response to ICI.The combination of DDR gene mutation and TMB is likely to be predictive.
DDR genes	Mariathasan et al. [42], Powles et al. [43]	Mutations in DDR pathway genes were associated with improved outcomes in exploratory analyses of both the IMvigor210 and JAVELIN Bladder 100 trials.	
Gene expression	Mariathasan et al. [42]	In IMvigor210, both a TGFβ ligand (TGFB1) and a TGFβ receptor (TGFBR2) were associated with nonresponse and reduced OS to ICI.	
TGFβ response signature (F-TBRS)		F-TBRS was associated with response in immune-excluded tumors.	
	Galsky et al. [44]	A higher F-TBRS signature was also associated with worse OS with atezolizumab in the IMvigor130 trial.	

PD-L1 is expressed in 20–30% mUC patients [27][45][46]. In BC, it is both a prognostic (increased expression PD-L1 by 20–30% correlates with advanced stage and worse outcomes) and a predictive marker for response to anti-PD-1 and anti-PD-L1 therapy [47]. Meta-analyses of prospective trials showed that, overall, PD-L1 expression is associated with radiographic response to ICIs in mUC patients [39][40]. At the same time, benefits from ICIs occur, regardless of PD-L1 expression. However, even among PD-L1-positive patients, single-agent ICI response rates are low and variable across randomized trials, ranging from 20% to 40% [35][36][48][49]. Although most trials analyzed the data using a prespecified cutoff for PD-L1 on IHC, the results did not consistently show improved responses with higher PD-L1 expression, which is not a very surprising observation, given that PD-L1 assays are not uniform across clinical trials (nonuniformity in the assays or scoring). While pembrolizumab and nivolumab clinical trials used the DAKO assays, Ventana assays were used for durvalumab and atezolizumab. In the pembrolizumab and nivolumab trials, PD-L1 tumor cell staining was used, whereas the IM vigor trial used PD-L1 immune cell staining.

The cutoffs for PD-L1 staining were also different. Variability in the staining platforms and cutoffs, including cell types and scoring system, may have been responsible for variability in the observed responses with different immune checkpoint inhibitors [46]. Other important factors which should be considered in using PD-L1 as a standalone biomarker to assess response to immunotherapy is the intratumoral heterogeneity with regard to PD-L1 expression and its dynamic nature in space and time during the disease course [47][50]. Attempts were made to harmonize PD-L1 assays in non-small-cell lung cancer and found consistent staining across some assays, but not with the others [51][52]. Limited inter-observer reliability in scoring PD-L1 staining on immune cells was also described [53]. The application of liquid biopsy and immune-targeting tracers for positron emission tomography (ImmunoPET) [54][55][56], may be a possible and more efficient way for serial monitoring of PD-L1 or other ICI biomarkers.

4.2. Tumor Mutation Burden

The tumor mutational burden (TMB) is defined as the total number of mutations per coding area of a tumor genome. A higher number of mutations increase the chances of generating neo-tumor-antigens, which can be recognized by the host immune system as immunogenic neoantigens [57][58][59]. TMB is quantified as the number of coding somatic mutations per megabase (MB) of DNA [58]. Tumors with high TMB have been demonstrated to have a microenvironment rich in immune cells and associated cytokines [60]. Bladder cancer is the most highly mutated cancer [61]. TMB has been linked to ICI response in mUC [42][62][63]. Pembrolizumab is approved as a therapeutic option across solid tumors with TMB ≥10 mutations/Mb without satisfactory treatment alternatives [64]. In the IMvigor210 trial, TMB assessed by targeted genomic profiling of 315 cancer-related genes (Foundation Medicine) correlated with a longer OS and ORR with atezolizumab independent of PD-L1 expression. Patients whose tumors had the highest mutation load (≥16/MB)) had a significantly longer survival compared with patients whose tumors had lower mutational loads (<16/MB) [HR 0.37, (95% CI 0.21–0.64)] [22][65]. TMB did not correlate with PD-L1 expression; however, it may be useful as an adjunct to other biomarkers in predicting outcomes with ICIs [66]. The combination of TMB and PD-L1 may be more efficacious together than either biomarker alone in predicting response to ICI [44][67]. Mutational signatures (denoting underlying tumor mutation) attributed to the APOBEC family of cytidine deaminases are frequently seen in BCs [28][68]. These were predictive of favorable responses to ICIs in mUC [40][42][44]. Recently, a meta-analysis across multiple cancer types including mUC suggested that clonal TMB (in all cancer cells in the clone) followed by total TMB was most predictive of response to ICIs. In addition, clonal TMB and the APOBEC signature were among the most important features associated with response on multivariate analysis [40]. As with PD-L1, selecting an optimal cutoff and harmonizing assays have been the common challenges in implementing TMB as a biomarker. In addition to the quantity (cutoff), the quality of the mutations (short insertions/deletions), clonality (clonal versus sub clonal), and the association of the neo antigens with the patient's HLA may be considered while assessing TMB as a biomarker [40].

4.3. Molecular Subtypes of Bladder Cancer

Basal type tumor cells have higher PD-L1 expression [69]. In the IMvigor 210 trial with atezolizumab, the luminal cluster II subtype had a statistically significant higher response rate compared to luminal cluster I, basal cluster I,

and basal cluster II subtypes [7]. Combining the Lund molecular classification scheme with TCGA scheme could lead to better prediction of responses. Tumors that were both genomically unstable (GU) in the later classification and luminal II had high TMB and better responses to ICI. On the contrary, tumors that were luminal II but not GU had low TMB and lower responses [42]. In the CheckMate 275 trial with nivolumab, improved responses were seen in basal I subtype followed by luminal II subtypes [70]. In the JAVELIN Bladder 100 trial of maintenance avelumab, however, there was no association between the TCGA subtypes and OS [34]. There is, thus, a heterogeneity in the outcomes with immunotherapy with respect to molecular subtypes. It may be plausible that the current molecular classifications of UC may not be adequately representative of appropriate molecular signatures predictive of response to ICIs; hence, further research is needed.

4.4. Gene Expression Profiling of the Tumor and Microenvironment

Tumor immunity is the result of a complex interaction between the tumor cells and immune cells in the tumor microenvironment (TME). A comprehensive immune gene expression profiling of these cell types, along with their chemokine and cytokine repertoire, may represent the ongoing interactions resulting in tumor immunity. As gene expression profiling is a dynamic display of ongoing cellular processes in the tumor and cells in the TME, it is more reflective of the molecular pathways involved at the time of sampling [27]. Two broad categories of gene expression signatures have been linked to ICI response in prospective mUC cohorts: a group of genes reflecting cytotoxic T-cell activity associated with ICI response; a group of genes reflecting immunosuppressive stromal signaling associated with ICI resistance. These signatures remain exploratory pending validation in additional prospective cohorts [27]. A variety of inflammatory gene signatures reflecting $CD8^+$T-cell activity and/or interferon-gamma signaling have been associated with ICI response in mUC. Some recurrent genes in these signatures include *CCL5*, *CD27*, *CD8A*, *CXCL9*, *CXCL10*, *CXCR6*, *GZMA*, *GZMB*, *IDO1*, *IFNG*, *LAG3*, *PRF1*, *STAT1*, and *TBX21* [27]. In the IMvigor 210 trial in mUC, a higher $CD8^+$T effector signature (PD-L1 positivity on the immune cells was associated with the expression of genes in a $CD8^+$T effector set) correlated with higher complete response rates to atezolizumab. Similarly, CXCL-9 and CXCL-10 (chemokines representative of the T effector signature) expression had a higher response to immunotherapy [42]. Notably, CXCL9 expression was one of the strongest predictors of ICI response in the Litchfield et al. meta-analysis of ICI biomarkers across tumor types [27]. In the Checkmate 275 study, a higher value of 25-gene interferon-gamma (IFN-γ) signature was associated with a higher response to nivolumab [70]. While IFN-γ is known to have favorable effects on antitumor immunity, persistent signaling has been associated with adaptive resistance to checkpoint therapy. One of the most important IFN-γ mediated effects is the increased expression of PD-L1 and PD-L2 [71]. Prolonged exposure of cancer cells to IFN-γ signaling leads to expression of a number of ligands for T-cell inhibition, which in turn leads to resistance to ICIs independent of the PD-1/PD-L1-pathway [72]. An eight-gene subset of that signature focused on CD8 T effector activity was positively associated with response in IMvigor210 [42]. TGF-β signaling in the tumor stroma creates an immunosuppressive phenotype or immune-excluded phenotype in that the cytotoxic T cells are separated from the tumor cells by a dense fibrous stroma, promoting angiogenesis and metastases. On the basis of data from the IMvigor210 study, Mariathasan et al. showed that increased pan fibroblast TGF-β response signature (F-TBRS), TGF-β ligand (TGFB1), and a TGF-β receptor (TGFBR2) in fibroblasts within the peritumoral stroma were associated with a lack of response and poorer survival to atezolizumab, especially in patients where $CD8^+$ T cells were excluded from the

tumor parenchyma [42]. A higher F-TBRS signature was also associated with worse OS for patients treated with atezolizumab rather than platinum chemotherapy in the IMvigor130 trial [44].

5. Biomarkers for Targeted Therapy in UC

The basis of targeted therapy is the specificity of treatment directed against a target that is preferentially altered in the cancer cells as compared to the normal cells. Three such targeted therapies have been approved in mUC, although many other targets have been evaluated but not yet approved. The targeted therapeutic molecules approved in UC include *FGFR* inhibitor erdafitinib, Trop 2 inhibitor sacituzumab govitecan, and Nectin-4 inhibitor enfortumumab vedotin (EV). Each of these molecules is approved in the second-line setting after progression on first-line platinum/non-platinum-based chemotherapy. As of now, only erdafitnib is recommended on the basis of the *FGFR* alteration status (mutation/fusion); thus, *FGFR* alterations serve as a biomarker for benefit from *FGFR* inhibitors. The other two molecules are approved irrespective of the biomarker results. There are no head-to-head trials comparing second-line chemotherapy, immunotherapy, and targeted therapy. Historically, responses with second-line chemotherapy have been dismal at around 10% with a median survival of 7–9 months [8][73][74]. Even in the second line, the response rates with immunotherapy are to the tune of 13–20% with amedian OS of 10 months [37][48][70][75][76]. In that context, most of these targeted therapies have been approved on the basis of their superior response rates as compared to the historical results, although the overall survival also compares favorably with immunotherapy. Erdafitinib, a pan-FGFR-kinase (FGFR 1–4) inhibitor, was approved in 2019 for patients with locally advanced or mUC with progression after platinum-based chemotherapy with known susceptible *FGFR2/3* alterations. The specific alterations include *FGFR3* mutations or *FGFR2/3* gene fusions. The approval was based on a phase II trial demonstrating overall response rates of 30–40% in a biomarker-driven population [77][78]. EV consists of a monoclonal antibody specific for Nectin-4 conjugated to monomethyl auristatin E (MMAE), a microtubule-disrupting agent [79][80]. Approval was also granted for locally advanced or mUC after prior platinum-based chemotherapy and ICI as a result of the phase II EV-201 trial. In the confirmatory phase III EV-301 trial, EV conferred a significant survival benefit over standard chemotherapy in the post-chemo/post-ICI setting, leading to regular FDA approval [81]. Notably, EV has shown benefit and is approved for treatment without regard to Nectin-4 levels. Considering recent data supporting a maintenance strategy with avelumab after platinum-based chemotherapy for advanced UC, as well as results from the EV301 study, EV may be a reasonable option at the time of the first relapse after maintenance immunotherapy [34]. EV in combination with pembrolizumab has been accorded a breakthrough therapy designation as first-line treatment for metastatic disease on the basis of a higher response rate and duration of response [82][83]. Regimens containing EV are being evaluated in the first-line (ClinicalTrials.gov numbersNCT04223856andNCT03288545) and perioperative (NCT03924895) settings. The third targeted therapy approved in mUC is SG (a monoclonal antibody specific for Trop-2 conjugated with SN-38), the active metabolite of irinotecan [84]. Approval for SG was recently granted in April 2021 after the phase II TROPHY-U-01 trial. This demonstrated a 27% ORR and 10.9 months median OS in the post-chemo/post-ICI setting [85]. Similar to EV, SG has been tested and approved without regard to the levels of its target, Trop-2.

5.1. FGFR

FGFR alterations are ubiquitous in UC. The most common FGFR3 alterations are mutations which account for 80% of the FGFR alterations in NMIBC and almost half of the alterations in MIBC [86][87]. FGFR alterations occur in 20% of the patients with advanced urinary bladder UC and up to 37% of the upper tract (UT) UC [88][89]. Of these, *FGFR3* alterations (mutations and fusions) are significant from a therapeutic perspective and are more common in UTUC than UBC [90][91]. Of the *FGFR3* mutations, S249C is the most common, accounting for up to half of these mutations [77][92]. *FGFR 2/3* mutations are enriched in the luminal type 1 molecular subtypes of UC, which are usually immune-excluded. These tumors show reduced T-cell infiltration, as well as low PD-L1 expression on TILs; hence, they are postulated to be resistant to immunotherapy [77]. Mutations in *FGFR*, which belongs to the family of tyrosine kinase receptors, bestow the cancer cells a clear survival advantage in that the receptor functions in a ligand-independent manner and the constitutive tyrosine kinase activity leads to incessant downstream signaling via the *RAS/MAP3K/PI3K* pathway, ultimately leading to cell proliferation [93]. *FGFR* fusions and amplifications are less common alterations in the *FGFR* pathway [94]. In the BCLC 001 trial of erdafitinib, up to three-fourths of the alterations were *FGFR2/3* mutations, and the remaining were *FGFR2/3* fusions [77]. As with driver mutations in lung cancer, it has been postulated that immunotherapy may not be an appropriate option for *FGFR* mutated UC. Data show that, in the immune-excluded luminal type 1 UC, limited responses are seen with immunotherapy [70][95]. In this subset enriched with *FGFR* alterations, durable responses have been seen with *FGFR* inhibitors after progression on immunotherapy [77][96]. On the contrary, the pivotal second-line immunotherapy trials in UC have shown responses irrespective of the *FGFR* alteration status, thus putting to question the notion that *FGFR* alterations are a biomarker for lack of response to immunotherapy [97]. A longer median duration of response (68% of patients with a response for at least 12 months) with fewer toxic effects of grade 3 or more (15% vs. 46%) suggests that immunotherapy may provide a better safety and efficacy profile than *FGFR* targeted therapy [37]. Thus, the optimal sequencing of therapy in *FGFR*-mutated mUC is debatable.

5.2. Nectin-4

Nectin-4 is a cell-adhesion molecule that is highly expressed in UC and may contribute to tumor-cell growth and proliferation [79][98]. EV consists of a monoclonal antibody specific for Nectin-4 conjugated to monomethyl auristatin E (MMAE), a microtubule-disrupting agent. The delivery of the microtubule payload into the tumor cells leads to cell-cycle arrest and apoptosis [79][80]. According to the phase III randomized controlled trial, EV significantly prolonged survival as compared with standard chemotherapy in patients with locally advanced or mUC who previously received platinum-based treatment and a PD-1 or PD-L1 inhibitor [81]. However, Nectin-4 expression was not mandatory for enrolment as high expression was observed in a vast majority of patients with advanced UC [98][99].

5.3. Trop 2

Erdafitinib is limited to patients with *FGFR2/3* mutation or fusion [100]. Many patients will still need newer therapies. Trophoblast cell-surface antigen 2 (Trop-2) is a transmembrane cell surface glycoprotein that is expressed extensively on most carcinoma cells and plays an important role in cell transformation and proliferation [101][102]. Thus, increased expression is associated with poor outcome, including mUC [84]. Sacituzumab govitecan (SG) is a

Trop-2-directed molecule composed of an anti-Trop-2 humanized IgG monoclonal antibody coupled to SN-38, the active metabolite of the topoisomerase 1 inhibitor irinotecan with a high drug-to-antibody ratio (7.6 molecules of SN-38 per antibody) [103]. Internalization of Trop-2-bound SG delivers SN-38 inside tumor cells, thus killing the tumor cells, whereas the hydrolyzable linker enables SN-38 to be released into the tumor microenvironment, killing adjacent cells (bystander effect) [104][105]. Approval for SG was recently granted in April 2021 after the phase II TROPHY-U-01 trial, and it demonstrated a 27% ORR and 10.9 months median OS in the post-chemo/post-ICI setting [85]. Similar to EV, SG has been tested and approved. A benefit with SG was even observed in a small subgroup with prior exposure to EV. Responses in patients previously treated with EV suggest various nonoverlapping mechanisms of action and resistance between the two antibody–drug conjugates [84]. The results from this phase II trial will be corroborated in the ongoing phase III confirmatory trial of SG versus taxane or vinflunine in mUC (TROPiCS-04; ClinicalTrials.govidentifier NCT04527991). Additional cohorts of TROPHY-U-01 continued to evaluate the role of SG in mUC. Cohort 2 is investigating the role of SG in platinum-ineligible patients with mUC who progressed after immunotherapy. Cohort 3 is evaluating SG in combination with pembrolizumab in patients with mUC who progressed after prior platinum-based chemotherapy and are immunotherapy-naïve. Both cohorts 4 and 5 are evaluating SG as induction and maintenance therapy in mUC patients who responded to induction platinum-based chemotherapy in the neoadjuvant setting, comprising chemotherapyeither alone (cohort 4) or in combination with avelumab (cohort 5) [84].

5.4. Other Targeted Therapies

HER2 is amplified in a subset of patients with UC. *HER2* amplification is an adverse prognostic event in UC. Yet, anti-HER2 therapy has not been proven to improve outcomes in mUC. One of the reasons is the heterogeneity in HER2 testing in trials with anti-HER2 therapy. In a retrospective analysis performed by a group, comparing HER2 IHC and *HER2* FISH results demonstrated that ASCO/CAP HER2 testing guidelines for breast cancer could be implemented in UC [46]. Hence, patients with true amplification of HER2 can be evaluated in future clinical trials utilizing anti-HER2 therapy in UC.PARP inhibitors have not been found to improve outcomes in *BRCA*-mutated/*HRR*-deficient UC [106]. The incidence of *BRCA1/2* mutations is about 1.5% and 1.4%, respectively, in UC [29]. Previously, it has been mentioned that defects in the DDR pathway may predict responses to chemotherapy. An innovative approach could be the combination of chemotherapy and PARP inhibitors in DDR-deficient UC. Other therapies that target *PI3K/AKT/mTOR*, *MAPK*, and *VEGF* pathways have also been investigated in UC. The RANGE study found that ramucirumab, a monoclonal VEGFR-2 antibody with docetaxel, improved the progression-free survival, but not OS in previously treated mUC patients [107].

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