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Genitourinary Cancers (S Gupta, Section Editor)

Role of Targeted Therapies in Management of Metastatic Urothelial Cancer in the Era of Immunotherapy

Petros Grivas, MD, PhD[1,2,3,4,*]
Evan Y. Yu, MD[1,2,3,4]

Address
[*,1]Division of Oncology, Department of Medicine, University of Washington, Seattle, WA, USA
Email: pgrivas@uw.edu
[2]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA
[3]Genitourinary Cancers Program, University of Washington, Seattle, WA, USA
[4]Seattle Cancer Care Alliance, 825 Eastlake Ave E, G-4830, Seattle, WA, 98109, USA

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Opinion statement

Despite significant advances and the approval of immune checkpoint inhibitors, metastatic urothelial carcinoma (mUC) is still very hard to treat and has poor outcomes. Deeper understanding of the molecular underpinnings of mUC has identified potential biomarkers, biologic drivers, and relevant therapy targets. However, targeted therapies in mUC have had significant challenges due to molecular heterogeneity, clonal evolution, and genomic instability, and have not improved outcomes so far. Despite that, recent technological developments, clinical utilization of molecular biology, and discovery of new agents with preclinical and early clinical activity have signaled a new age of experimental therapeutics in mUC. The more frequent use of next-generation sequencing of tumor tissue and cell-free circulating tumor DNA, combined with novel agents tested in clinical trials, provides promise. There is a plethora of agents being tested in mUC, including inhibitors of receptors and signaling pathways (e.g., fibroblast growth factor receptor, human epidermal growth factor receptor, phosphatidylinositol 3-kinase/AKT/mTOR pathway), angiogenesis (e.g., vascular endothelial growth factor and its receptors), poly (ADP-ribose) polymerase (PARP) inhibitors, immuno-oncology agents, cytotoxic agents (e.g., chemotherapy, antibody drug conjugates), and epigenetic modulators, among others. Two agents, enfortumab-vedotin (antibody drug conjugate) and erdafitinib (fibroblast growth factor

receptor inhibitor), have breakthrough designation by the FDA, but are not approved as of March 2019. Novel combinations with various modalities and optimal sequencing of active therapies are being investigated in clinical trials. More sophisticated patient selection, discovery and prospective validation of predictive (and prognostic) biomarkers with clinical utility, and relevant clinical trial designs are important parameters in that context. Based on the above, there is high potential for targeted therapies to be added in the growing armamentarium of mUC, and possibly complement chemotherapy and immuno-oncology agents.

Introduction

Bladder cancer (BC) very common in the USA with estimated 81,190 new cases and 17,240 deaths in 2018 [1]. It is more common in men (about 3:1) and Caucasians. Urothelial carcinoma (UC) is the most common type of BC and can ascend from the entire urinary tract, e.g., renal pelvis, ureter, and urethra. Metastatic urothelial carcinoma (mUC) is treated the same way, regardless of the initial anatomic site of origin. Conventionally, platinum-based chemotherapy has been the cornerstone of first-line (1 L) therapy; however, in the last few years, immune checkpoint inhibitors (CPIs) against programmed cell death protein-1 (PD-1) and programmed death ligand-1 (PD-L1) have shown durable responses in 1-L and salvage treatment settings [2]. Overall survival (OS) benefit has also been demonstrated over salvage single agent taxane or vinflunine chemotherapy in platinum-refractory mUC [3••, 4•]. Despite durable activity in many patients, most unfortunately do not respond. Additionally, therapy-related adverse events can impact the quality of life and occasionally can be severe. Moreover, there is significant cost to healthcare systems associated with the use of these agents [5].

The prognosis of mUC remains overall poor, and there is an unmet need for new, safe, and effective therapies. Deeper understanding of the molecular profile of mUC can identify putative predictive biomarkers and therapeutic targets. Those advances, in conjunction with emerging novel compounds that target specific key molecules in cancer cells and the tissue microenvironment, provide a solid foundation for the increasing utilization of targeted therapeutic approaches in mUC clinical trials. In this article, we discuss updates in the molecular context of UC, setting the conceptual framework, and we review emerging targeted therapies that have shown promise, summarizing examples of clinical trials. We also outline relevant considerations in the overall effort to improve outcomes using targeted therapies in mUC. Since the field is very dynamic, this review is not meant to be exhaustive, but rather to cover the major mechanisms under investigation at this time point.

Updates in the molecular landscape of UC

Multiple studies have attempted to designate molecular subtypes based on gene expression profiling, and fit those advances into classification schemes, such as those demarcated by the UROMOL, University of North Carolina (UNC), MD Anderson Cancer Center (MCACC), The Cancer Genome Atlas (TCGA), and Lund University (LU) studies [6–9,10•,11,12,13••,14,15]. This molecular dissection has provided classification of UC into subtypes with distinct transcriptomic outlines with variance in response to specific treatment types.

Overall, luminal and basal subtypes were described as separate forms of muscle-invasive bladder cancer (MIBC). Basal MIBC subtypes harbor more RB1 and NFE2L2 mutations, whereas luminal subtypes exhibit more frequent

alterations in FGFR3 and KDM6A genes [8–12,13••]. Interestingly, genomic alterations in the luminal subtypes resemble non-muscle invasive bladder cancer (NMIBC) that may have progressed to a more advanced stage. Moreover, GATA3, PPARγ, and FOXA1 expression are characteristics more suggestive of luminal papillary subtype [12,13••].

In the TCGA project including 412 MIBC tumors, five molecular subtypes were recognized [10•, 13••]. Those were described as luminal papillary, luminal infiltrated, luminal, basal squamous, and neuronal. Discordance in OS was shown within those subtypes and was associated with tumor mutational burden (TMB). The luminal papillary subtype had higher mutational rate and the longest 5-year OS. The most common mutations were TP53 (48%), KMT2D (28%), KDM6A (26%), ARID1A (25%), and PIK3CA (22%), whereas amplification was most common in E2F3, PPARγ, and MDM2; CDKN2A deletions were frequent (22%), and FGFR3 fusions were noted in 2%. Epigenetic analyses along with gene expression acknowledged 158 genes that were silenced. Microbial analysis found HPV genomic integration in a very small proportion of tumors (2.6%). The main signaling pathways most frequently affected were cell cycle regulators (89%), PI3K pathway (72%), and chromatin remodeling, especially histone-modifying factors [10•,13••].

In another analysis of 295 cases of mUC, a comprehensive genomic profile showed several significant alterations, e.g., CDKN2A (34%), FGFR3 (21%), PIK3A (20%), and HER2 (ERBB2) (17%) [14]. Comparable findings were found in upper tract UC (UTUC) samples. A study compared upper and lower tract UC showing comparable alterations, but with different frequency [15]. For instance, FGFR3 was more frequent in UTUC (35.6% vs. 21.6%, $p = 0.065$); the same was found for CDKN2B alterations (15.3 vs. 3.9%, $p = 0.016$).

Besides tumor somatic alterations, there is interesting data about germline mutations in UC [16, 17]. A study of patients with UC with germline mutations that have known cancer predisposition potential, including DNA damage response (DDR) genes, noted a high prevalence of pathogenic variants. Alterations in DDR genes, including BRCA 1 and 2, ATM, MSH2, PALB2, and CHEK2, among other genes, were noted in 29% of patients with UTUC and 13% of those with lower tract UC [16]. Moreover, the data provided information pertaining to the broader family members with potential for cascade testing; 9% of patients had family history of UC, while 19% had history of other cancers. The results highlighted the critical role of genetic counseling and germline testing in UC, especially in patients with young age of cancer diagnosis, patients with suspicious personal and/or family history of cancer, and patients with UTUC. In addition, in the context of prevention, it is important to involve genetic counselors to review tumor somatic testing that may divulge germline mutations; it is important to recognize that tumor somatic testing is not destined to replace germline testing [18, 19]. It is noteworthy that deleterious DDR alterations have been associated with response to platinum-based chemotherapy, anti-PD-1/PD-L1 agents, and poly (ADP-ribose) polymerase (PARP) inhibitors (to be discussed later).

Furthermore, cell-free circulating tumor (ct) DNA testing may also be employed for UC profiling. In a study of 369 patients with mUC with ctDNA next-generation sequencing (NGS) testing, a plethora of genomic alterations was noted, which seemed comparable to prior studies of UC tumor tissue [20]. Alterations comprised ARID1A, TP53, PIK3CA, HER2, and FGFR3, among

Table 1. (a) Examples of ongoing trials evaluating targeted agents as first line therapy in mUC and (b) examples of ongoing trials evaluating targeted agents in the salvage setting of mUC

Phase	Sample size (*N*)	Therapy	Endpoint	NCT no.
(a)				
III	500	GC + (placebo or bevacizumab)	OS	NCT00942331
II	150	Durvalumab ± olaparib	PFS	NCT03459846
II	39	Pembrolizumab + cabozantinib	ORR	NCT03534804
II	40	Avelumab + axitinib	ORR	NCT03472560
Ib	30	Atezolizumab + cabozantinib	ORR	NCT03170960
III	694	Pembrolizumab + (lenvatinib or placebo)	OS/PFS	NCT03898180
(b)				
III	631	Erdafitinib vs. vinflunine or taxane or pembrolizumab based on FGFR genomic alterations	OS	NCT03390504
III	400	Rogaratinib vs. chemotherapy based on FGFR gene overexpression	OS	NCT03410693
II	300	Docetaxel + placebo vs. docetaxel + vofatamab vs. vofatamab post checkpoint inhibitor based on FGFR genomic alterations	PFS	NCT02401542
II	125	Debio 1347	ORR	NCT03834220
II	35	Regorafenib	PFS	NCT02459119
II	200	Rucaparib	ORR	NCT03397394
II	139	Rucaparib + nivolumab (allows first-line cisplatin-ineligible)	ORR	NCT03824704
II	60	Olaparib based on DDR alterations	ORR	NCT03375307
II	30	Everolimus based on TSC1, TSC2, mTOR alterations	ORR	NCT02201212
II	209	Sapanisertib based on TSC1, TSC2 mutations	ORR	NCT03047213
II	95	Afatinib	PFS	NCT02122172
Ib	196	Durvalumab ± AZD4547/olaparib/ AZD1775/vistusertib/AZD9150/selumetinib	Toxicity	NCT02546661 (BISCAY)
Ib	152	Cabozantinib + nivolumab ± Ipilimumab	ORR	NCT02496208
Ib	30	Atezolizumab + cabozantinib	ORR	NCT03170960
I	65	Rogaratinib + copanlisib based on FGFR gene overexpression	Toxicity	NCT03517956
II	200	EV	ORR	NCT03219333
III	550	EV vs. docetaxel or paclitaxel or vinflunine	OS	NCT03474107
I	159	EV + pembrolizumab EV + Cis EV + Carbo EV + gemcitabine EV + pembrolizumab + Cis or Carbo	Toxicity	NCT03288545
I/II	500	SG	Toxicity/ORR	NCT01631552
II	140	SG	ORR	NCT03547973
I	99	Trastuzumab deruxtecan + nivolumab	Toxicity/ORR	NCT03523572
I	70	PRS-343 + atezolizumab	Toxicity/ORR	NCT03650348
I	78	PRS-343	Toxicity	NCT03330561
I/II	53	Guadecitabine + atezolizumab	Toxicity, ORR	NCT03179943

Unselected patients unless noted in treatment column. AZD4547: FGFR inhibitor; AZD1775: WEE1 inhibitor; vistusertib: mTORC1/2 inhibitor; AZD9150: Stat3 antisense oligonucleotide; selumetinib: MEK inhibitor

GC gemcitabine/cisplatin, *N* sample size, *Carbo* carboplatin, *Cis* cisplatin, *CR* complete response, *DCR* disease control rate, *DDR* DNA damage response, *DFS* disease-free survival, *EV* enfortumab-vedotin, *ORR* overall response rate, *OS* overall survival, *PFS* progression-free survival, *SG* sacituzumab govitecan, *TURBT* transurethral bladder tumor resection

others. A more recent study using ctDNA confirmed prior findings and, also, identified RAF1 and BRCA1 as putative prognostic biomarkers in mUC [21]. The concordance between tumor tissue and ctDNA is being evaluated in UC studies [22]. Additional molecular studies are continuing, and the high rate of alterations supports further the evaluation of targeted therapeutics in mUC. The potential clinical utility of molecular biomarkers, e.g., PD-L1 protein expression, and FGFR mutations/fusions, for which pertinent therapies are available, is the objective of current studies.

Available findings set a solid basis for the prospective evaluation of targeted therapies in mUC, utilizing precision oncology and systems biology approaches in target discovery and biomarker-based patient selection. A detailed explanation of the concepts of precision oncology and biomarker development in UC has been delineated in prior reviews [23, 24], while the field has been rapidly evolving with both clinical trials and translational studies. In the next sessions, we summarize a number of promising examples of targeted therapies being evaluated in mUC, along with ongoing clinical trials (Table 1).

Antibody-drug conjugates

Antibody-drug conjugates (ADC) are targeted antibodies combined with cytotoxic drugs via a linker molecule. Enfortumab-vedotin is an ADC, targeting the highly expressed Nectin-4 with an antibody linked to MMAE. In a phase I trial, patients with mUC treated with prior chemotherapy or who were unfit for cisplatin received enfortumab-vedotin with acceptable tolerability (fatigue was the most common adverse event) [25••]. Overall response rate (ORR) was 33% (95% CI 24.7–42.9) in the initial report, but reached 40% at the time of the oral presentation at the 2018 Annual ASCO Meeting. The overall median response duration was 24.3 weeks (95% CI 16.3–47.3) and progression-free survival (PFS) 23.1 weeks (95% CI 20.1–24.1). Median OS was 12.5 months (95% CI 8.1–14.8) with 68% of patients censored, and 6-month OS was 75.1%. Phase II trial data will be presented very soon, while a recent Press Release announced that the trial met its primary endpoint of ORR (44%). Currently, a phase III trial (NCT03474107) is ongoing in mUC, randomizing 550 patients with previously treated mUC to enfortumab-vedotin vs. salvage chemotherapy (taxane or vinflunine) with OS being the primary endpoint (NCT03474107). This agent is also being evaluated in combinations with chemotherapy and pembrolizumab in a separate phase Ib trial (NCT03288545). It received breakthrough designation status by the FDA, but is not yet approved as of March 2019.

Another promising ADC is sacituzumab govitecan (IMMU-132), which links the cytotoxic agent SN-38 to an anti-Trop-2 antibody. A recent 45-patient phase I/II trial demonstrated ORR 31%, median PFS 7.3 months (95% CI 5.0–10.7), median OS 16.3 months (95% CI 9.0–31.0), and manageable toxicity in a previously treated mUC population [26]. A larger phase II trial with IMMU-132 in patients with previously treated mUC is ongoing (NCT03547973).

ASG-15ME was tested in a phase I trial in 49 patients with mUC [27]. Of the 43 patients evaluable for response at the time of presentation, 14 had ORR, including a complete response; disease control was achieved in 27 patients. Most common treatment-related adverse events of any grade included fatigue, nausea, and peripheral neuropathy.

Trastuzumab deruxtecan, DS-8201a, is an ADC consisting of trastuzumab and DXd, a new topoisomerase-I inhibitor with promising results in HER2-positive gastric and breast cancers [28]. Due to the noted prevalence of HER2 amplification in UC (11% per TCGA), a combination DS-8201a and nivolumab is being evaluated in a phase Ib trial of patients with HER2-expressing breast and urothelial tumors (NCT03523572).

Fibroblast growth factor receptor pathway

Fibroblast growth factor receptor (FGFR) gene amplification and overexpression can result in cancer development, via multiple mechanisms, including cell growth, angiogenesis, and can mediate resistance to treatments [29]. There exist four FGFR receptors (FGFR1–4); FGFR mutations, fusions/rearrangements, amplification, and overexpression have been described as predictive biomarkers and treatment targets with stimulating results across tumor types [29].

FGFR3 mutations have been described in UC (more frequent in NMIBC and UTUC), and have been proposed to have a prognostic role. In a large study of patients with NMIBC, FGFR3 mutations were associated with better outcomes [30]. In another study, FGFR3 mutations were assessed along with expression of MIB-1, TP53, and P27KIP1 [31]. Patients with FGFR3 mutations and low MIB-1 expression had more favorable prognosis compared to patients with non-mutated FGFR3 and high MIB-1 expression (worst prognosis) or patients with non-mutated FGFR3 and low MIB-1 expression (intermediate prognosis).

Phase I trials have noted anti-tumor activity with FGFR inhibitors across tumor types, related to FGFR alterations and/or overexpression. Erdafitinib (JNJ-42756493) is a pan-FGFR receptor tyrosine kinase (RTK) inhibitor. In a phase I trial of this agent in advanced solid tumors (12% mUC), tolerability was noted [32]. Results from a phase II trial (BLC2001) showed notable efficacy of erdafitinib in mUC in patients with tumors harboring FGFR alterations [33••]. In that trial, 96 patients were treated with median of 5 cycles of erdafitinib; 10% were chemotherapy-naïve, 47% had received ≥ 2 prior lines of therapy, and 80% had visceral metastases. There was 42% confirmed ORR (3% CR, 39% PR) and 80% disease control rate. There was 70% confirmed ORR in 21 patients with prior CPI. Adverse events were manageable; 10% discontinued due to treatment-related events, but there were no treatment-related deaths. A large randomized phase III trial comparing erdafitinib vs. vinflunine or docetaxel, and erdafitinib vs. pembrolizumab, using FGFR mutations or fusions/translocations for patient selection is ongoing (NCT03390504). This agent is not approved as of March 2019, but has breakthrough designation by FDA.

Another pan-FGFR inhibitor is rogaratinib which is being tested in combination with the anti-PDL1 agent atezolizumab in the FORT2 trial (NCT03473756). Rogaratinib was evaluated in a phase I trial that used FGFR mRNA overexpression as the biomarker for patient selection [34]. In that trial, almost half of the samples exhibited a positive biomarker. ORR was 24%, suggesting that treatment based on FGFR mRNA overexpression merits evaluation; thus, a phase II/III trial comparing rogaratinib to chemotherapy in platinum-refractory mUC was initiated (NCT03410693).

Infigratinib (BGJ398) is another FGFR inhibitor that showed tolerability and promising activity in patients with FGFR-altered mUC in an early-phase trial; further assessment is being planned [35]. Another promising FGFR inhibitor in mUC is INCB054828. The phase II Fight-201 trial reported activity in patients who progressed after, or could not, receive platinum-based chemotherapy and had tumors exhibiting FGFR alterations [36]. Sixty-four patients had FGFR3 mutations with ORR of 25%. Additional FGFR inhibitors being evaluated in mUC include Debio1347 (NCT01948297), focusing on patients with tumors having FGFR fusions/translocations, AZD4547 (single agent and combined with durvalumab) in module A of the BISCAY trial evaluating FGFR mutations/fusions (NCT02546661), the antibody vofatamab/B-701 (NCT02401542), and others.

There are variations in the biomarkers employed across various trials (mutations/fusions vs. overexpression using different assays) that may result in different selected populations and conflicting ORR rates. This raises the point of which biomarker is more appropriate. Another question, relevant to patient selection, is the alleged value of FGFR gene amplification, especially with a high copy number that might serve as a potential tumorigenic driver; however, most trials in mUC have not included gene amplification as a selection biomarker. Furthermore, data propose that hyperphosphatemia may be a predictive biomarker of response to anti-FGFR agents, implying that intra-patient dose titration according to serum phosphate level may be a clinically relevant approach; this aspect can also be assessed in the larger ongoing trials [33••]. Nevertheless, this entails very close monitoring and available resources for continuous patient contact, evaluation, and dose adjustments in the outpatient clinical setting.

Interestingly, the UC luminal I papillary subtype is enriched for FGFR3 alterations [10•, 13••], which appear associated with low immune infiltration and poor responsiveness to CPIs. Many patients who may not respond to CPIs may later respond to FGFR inhibitors in clinical trials, raising the hypothesis of using molecular subtyping for patient selection; however, this concept merits further evaluation in prospective trials and translational studies. Moreover, the combination of FGFR and PD-(L)1 inhibition is being evaluated, given the potential for additive or synergistic efficacy in a molecularly heterogeneous, complex, and diverse disease (Table 1).

Vascular endothelial growth factor pathway

Vascular endothelial growth factor (VEGF) alterations and overexpression can result in angiogenesis which is an essential feature of tumor growth and treatment resistance [37]. This may be mediated by several factors, such as hypoxia-inducible factors (HIFs), matrix metalloproteinases (MMPs), and VEGF. VEGF expression was shown to correlate with advanced stage, progression, and recurrence across tumor types, including UC [38]. VEGF inhibitors have thus been tested in mUC across treatment lines.

The combination of ramucirumab (anti-VEGF-2) plus docetaxel has translated into a higher ORR (24.5% vs. 14%) and modest PFS over docetaxel/placebo (median 4.1 months [95% CI 2.96–4.47] vs. 2.8 months [2.60–2.96]; HR 0.757, 95% CI 0.61–0.94; $p = 0.0118$) after progression after prior platinum-based chemotherapy in the phase III RANGE trial ($N = 530$) [39].

However, there was no known OS benefit, which is very pertinent in this setting. Bevacizumab combined with 1 L gemcitabine/cisplatin was active in a phase II trial of 43 patients with mUC [40]. Complete response was noted in eight patients (19%) and partial response in 23 (53%) with 72% ORR; median PFS and OS were 8.2 and 19.1 months, respectively. Venous thromboembolic events (VTE) were concerning, but were mitigated by dose reduction of gemcitabine (8% had VTE after vs. 41% before dose reduction). Although the targeted goal of 50% improvement in PFS was not met, the promising data led to the CALGB 90601 (Alliance) phase III trial. This is a randomized, double-blind, placebo-controlled study comparing gemcitabine and cisplatin with bevacizumab or placebo as 1-L therapy in mUC; accrual was completed, and results will be presented very soon. Tyrosine kinase inhibitors, such as sunitinib and sorafenib, have shown unsatisfactory results in phase II trials in mUC, either as 1-L therapy, switch maintenance after 1-L therapy, or as salvage therapy [41–43]. Regorafenib inhibits the Tie-2 axis and other targets in addition to VEGFRs, and is being investigated in platinum-treated patients with mUC (NCT02459119). Cabozantinib which targets Met and Axl in addition to VEGFR2 has shown activity in pretreated patients, with relevant immune modulating activity when combined with CPIs, ipilimumab, and nivolumab in pretreated patients; accrual continues in expansion cohorts [44,45]. The combination of cabozantinib with either atezolizumab or pembrolizumab is also being pursued. Given the OS benefit established with the combination of VEGFR and PD-(L)1 inhibitors in other malignancies, such as renal cell carcinoma, there is interest for further assessment in mUC. For example, the combination lenvatinib/pembrolizumab is being compared to placebo/pembrolizumab as 1-L therapy of cisplatin-unfit patients in a phase III trial with PFS and OS as primary endpoints (NCT03898180).

In general, absence of patient selection based on clinically useful biomarkers may have negatively impacted the trials using VEGF inhibitors. However, several clinical trials are ongoing (NCT00942331, NCT03133390, NCT03272217) and provide the opportunity for exploration of presumed biomarkers that may correlate with treatment response. Biomarkers indicative of response to anti-VEGF therapies have been explored in various tumor types and may provide direction in mUC. Nevertheless, discrepancies in tumor cells and microenvironment across tumor types render the prospective validation of candidate biomarkers very hard.

Epidermal growth factor receptor pathway

Epidermal growth factor receptor (EGFR) gene amplification or mutations have been considered "targetable" alterations in many tumors, since the EGFR signaling pathway is involved in tumorigenesis [46, 47]. In a cohort of 599 UC samples, EGFR amplification was noted in 14% and was more common in basal UC [47]. EGFR amplification has been associated with more advanced UC and treatment resistance, being poised as a very relevant therapeutic target [46,47].

Several anti-EGFR agents have been prospectively investigated in UC, including cetuximab, panitumumab, gefitinib, and lapatinib, across different UC spaces and treatment settings [48–51]. Whereas EGFR amplification and

overexpression is frequent in mUC, most trials have shown modest results, which may be attributed to suboptimal patient selection and employment of protein expression by immunohistochemistry (IHC) rather than next-generation sequencing (NGS) testing. Moreover, concurrent aberrations in other signaling pathways may mediate de novo and/or acquired resistance, while tumor heterogeneity is also relevant. However, encouraging results with the pan-HER inhibitor afatinib in patients with tumors having HER alterations propose that patient selection based on NGS testing may be required when evaluating anti-HER agents (NCT02780687, NCT02795156, NCT02122172) [52].

Human epidermal growth factor receptor 2 pathway

Human epidermal growth factor receptor 2 (HER2) mutations, amplifications, and/or overexpression have been evaluated as potential prognostic and predictive biomarkers and treatment targets in breast and gastric/GEJ cancer, among other tumor types, including mUC [53, 54]. HER2 alterations were noted in 40% of micropapillary UC cases, while in other studies, HER2 amplification varied from 4 to 32% [55, 56]. A separate study suggested that luminal UC tumors had a significantly higher proportion of HER2 alterations compared to basal subtypes [57]. In addition, the presence of HER2 alterations correlated with higher TMB in mUC, supporting combination of anti-HER2 with CPI [58]. A recent review discussed in detail the HER2 inhibition potential in mUC [53].

Various HER2 inhibitors have been evaluated in mUC with mixed results. A phase II trial investigating trastuzumab, paclitaxel, gemcitabine, and carboplatin showed 70% ORR with HER2-positive mUC based on IHC, FISH, and/or elevated serum HER2 level, but there were a few cases of cardiac toxicity noted in that study [59]. A phase III trial assessing lapatinib vs. placebo as switch maintenance after 1-L chemotherapy in patients with HER2-positive tumors (by IHC) noted no difference between groups in median PFS (4.5 vs. 5.1 months, HR = 1.07, 95% CI 2.8–5.4, $p = 0.63$) [50]. Promising results were noted in a phase II trial of afatinib in platinum-refractory mUC: five out of 23 patients met the PFS endpoint, but all of them had tumors with HER alterations [52]. This resulted in the design of an ongoing larger trial of afatinib HER-positive mUC using NGS (NCT02122172).

The reason behind variances in response among tumor types is unclear, but may be related to other oncogenic alterations, e.g., PI3K pathway, MET, EGFR, HER3, which may mediate resistance to anti-HER2 monotherapy. HER2 alterations, as a therapeutic target, are being explored in the MyPathway "basket" trial (NCT02091141). One of the arms is evaluating the combination of trastuzumab with pertuzumab, which is a monoclonal antibody inhibiting the HER2/HER3 heterodimerization, in patients with chemotherapy-refractory solid tumors with HER2 overexpression (by IHC) and/or gene amplification. Early results in 12 patients with mUC showed that five patients had clinical benefit, with a complete response in a patient with metastatic peritoneal disease and two other partial responses [60]. The landmark NCI-MATCH trial (NCT02465060) is investigating numerous targeted therapy approaches, including three with anti-HER2

activity, such as afatinib (arm B), trastuzumab/pertuzumab (arm J), and ado-trastuzumab emtansine (arm Q).

Phosphatidylinositol 3-kinase /AKT/mTOR pathway

The phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR pathway is a very relevant signaling pathway with a clear role in tumorigenesis, cell cycle regulation, and growth [61–63]. Alterations are very common in mUC, while TCGA data suggest that this signaling pathway is deregulated in up to 72% of tumors [10•, 13••]. PIK3CA, target of rapamycin (mTOR), TSC1, and AKT can potentially be inhibited by compounds. The importance of this pathway was also outlined in a study of 103 samples, suggesting that targeted therapies focusing on the PIK3CA/AKT/mTOR pathway can generate high impact in mUC [64].

There is no high-level evidence regarding biomarkers predictive of PI3K pathway inhibition in mUC. However, TSC1/2 alterations could predict response to pathway inhibitors [65, 66]. TSC1 loss of function was critical in a phase II study of everolimus in mUC that did not meet its primary endpoint [67]. Nevertheless, robust response in a small subset of outlier patients led to analysis, showing that TSC1-inactivating mutations or deletions were associated with response to that mTOR inhibitor [66]. Such analyses could be a model for the development of other precision oncology targets. Notably, in an abovementioned trial with the FGFR inhibitor rogaratinib, PI3KCA and RAS mutations seemed to mediate resistance to that agent [34].

The phase II AUO AB 35/09 trial investigated the role of paclitaxel/everolimus combination as salvage therapy in mUC [68]. Patients with no prior response to platinum-based chemotherapy were treated with up to 6 cycles, but results were modest (median progression-free survival < 3 months and ORR 13%). The therapeutic premise in mUC is being evaluated in the module E of the BISCAY trial (NCT02546661) using AZD2014 combined with durvalumab.

Another potential use of mTOR inhibition is radio-sensitization. An in vivo and in vitro study tested radiation and mTOR inhibition alone and in combination, and noted an additive effect using the combination [69]. Cell cycle analysis showed that this effect was associated with cell cycle arrest in G1 and G2 phases. The potential of additive toxicity requires evaluation in clinical trials.

PARP inhibition

PARP inhibitors may inhibit tumor development by a number of mechanisms, including inhibition of base excision repair and PARP trapping. This resulted in the evaluation of PARP inhibitors in several cancers with BRCA1/2 mutations, including mUC [70, 71]. Early data have shown that several tumors with homologous DNA repair defects can be sensitive to PARP inhibitors, supporting further evaluation of PARP inhibitors to exploit the premise of synthetic lethality in mUC. This approach is being investigated in many clinical trials with PARP inhibitors either as single agents or combined with other agents (NCT03397394, NCT03375307, NCT03459846, NCT02546661). In one of those trials (ATLAS phase II, in previously treated patients with mUC), patients are being enrolled regardless of the presence of DDR gene alterations, based on observations of efficacy with rucaparib in patients with ovarian cancer without

DDR alterations, also in the context of the genomic instability seen in both tumor types. Genome-wide loss of heterozygosity (LOH) has also been proposed as a biomarker of genomic "scarring" and could potentially predict response to PARP inhibition.

Epigenetic modifiers

Genomic alterations in chromatin-modifying genes are commonly seen in mUC [10•, 13••]. CREBBP and EP300 inactivating mutations or deletions are noted in ~ 15% for each gene, which can result in dysregulated acetylation [72, 73]. Nevertheless, in nine evaluable heavily pretreated patients with mUC enriched with such alterations in a phase II trial assessing mocetinostat (selective histone deacetylase [HDAC] inhibitor), only one patient had response [73]. This very modest activity may have also been related to poor performance status and overall drug exposure, but resulted in early trial discontinuation. Additionally, vorinostat induced no response in a phase II trial of platinum-treated patients with mUC [74]. Therefore, the value of epigenetic-modifying therapies may warrant further assessment using rational agents in properly selected and less heavily pretreated patients; e.g., EZH2 inhibitors may warrant evaluation in tumors with KDM6A loss or ARID1A mutation [75, 76]. In addition, combination with CPIs may be beneficial and early trials testing those strategies are ongoing, e.g., atezolizumab combined with guadecitabine, a DNA methyltransferase inhibitor in patients who have progressed after the PD-(L)1 inhibitor. This agent is theorized to provoke viral mimicry and trigger epigenetic reprogramming of exhausted T cells (NCT03179943).

Other targeted agents

Considering the frequent alterations in retinoblastoma (Rb) pathway genes, palbociclib, a selective inhibitor of CDK 4/6 that restores Rb function and promotes cell cycle arrest, was investigated in a phase II trial [77]. However, despite rational selection of platinum-treated patients with mUC exhibiting p16 loss, but with intact Rb protein expression, palbociclib did not show significant activity. Combinations can still be worth pursuing, especially in molecular selected subsets based on preclinical and clinical data; the role of concurrent genomic alterations is also worth exploring. Other new agents are being explored in combination with the PD-L1 inhibitor durvalumab in the phase Ib BISCAY trial (Table 1), including a STAT3 antisense oligonucleotide (AZD9150), WEE1 inhibitor (AZD1775), and MEK inhibitor (selumetinib).

Cytotoxic chemotherapy

Alterations in DDR genes may have an important role in mUC [78]. A recent study investigated the functional impact of missense mutations in the nucleotide excision repair (NER) gene, *ERCC2*, and the association with response to cisplatin-based neoadjuvant chemotherapy [79]. This study validated previous clinical data further supporting the predictive role of ERCC2 inactivation mutations in this setting [80]. In addition, another study reported that alterations

in three other relevant genes (ATM, Rb1, FANCC) also predicted response to cisplatin-based neoadjuvant chemotherapy and longer OS [81]. There are three very important clinical trials trying to prospectively validate these findings and assess the clinical utility of DDR gene alterations in the neoadjuvant setting (NCT02710734, NCT03609216, NCT03558087). DDR gene alterations have been associated with better outcomes in platinum-treated patients with mUC, suggesting that the potential impact of DDR gene alterations may not pertain only to the neoadjuvant setting [82]. Moreover, DDR alterations have also been correlated with response to anti-PD-1/PD-L1 agents, along with other biomarkers in development [83]. The role of locus-specific or genome-wide LOH can also be explored in clinical trials, along with the functional impact of alterations, and their germline vs. somatic nature.

Challenges and future directions

Targeted therapies certainly have the potential to be included in the treatment armamentarium of mUC, but have not met the bar for approval as of March 2019, mainly due to lack of proper biomarker-enriched patient selection, molecular heterogeneity and pathway redundancy, and lack of drug efficacy, among other reasons. There are numerous identifiable genomic alterations, but many may not be "targetable" or major "disease drivers." Validation of biomarkers and treatment targets is a prerequisite for successful deployment of targeted therapies in mUC [24, 84].

There have been several strategies to evaluate the precision oncology strategy across tumor types. In the IMPACT trial, which investigated the efficacy of "matched" and "nonmatched" targeted therapy (1307 of 3743 tested), 10.4% of patients with "matched" targeted therapy reached the plateau of 38 months in median OS vs. 4% in the non-matched group ($p < 0.0001$), supporting that concept [85]. However, even with "matched" targeted therapies, the outcomes need to be better; the results merit validation, and the impact of precision oncology on outcomes is still unclear. In a retrospective study, genomic characterization impacted treatment in only 16% of patients, and treatment based on that profiling did not improve outcomes over non-directed therapy approach [19]. In the MOSCATO-01 trial, 199 patients were treated with "matched" therapy, including 15% with tumors with urological origin [86]. ORR was 11% (95% CI 7–17%) and median OS 11.9 months (95% CI 9.5–14.3). The SHIVA trial compared patients treated with targeted therapy with those treated according to physician choice (control) and showed median PFS of 2.3 months (95% CI 1.7–3.8) vs. median PFS of 2.0 months (95%CI 1.8–2.1) (HR 0.88, 95%CI 0.65–1.19, $p = 0.41$) [87]. Those trials suggested that while "actionable" genomic alterations exist across tumor types, the results may often be modest. The ongoing TAPUR (NCT02693535; supported by ASCO) and NCI-MATCH (NCT02465060; supported by NCI) trials will help state the role of precision therapy across cancers, while there are discussions about next versions of the NCI-MATCH trial utilizing several combinations of targeted therapies or CPIs.

An additional contest is the discordance in gene expression within histological subtypes in the same tumor. A variant in a tumor may result in dissimilar phenotypes. For instance, identical TP53 DNA rearrangements in a tumor were

associated with inconstant basal or luminal subtype expression depending on whether the sample was derived from the squamous or urothelial tumor component [88]. There is a need to evaluate further histologically divergent transcription events in mUC, since differential transcriptional regulation and post-translational modifications can affect phenotypes and treatment response. This is a very challenging concept to conquer, but deeper understanding of tumor biology over time is critical in this very heterogeneous cancer. A major challenge is the distinction between prognostic and predictive biomarkers; the latter is more specific to prediction of benefit from a specific therapy. However, both require clinical utility to be proven before incorporation into the clinical practice. There are examples of biomarkers that were thought to be predictive and ended up showing prognostic relevance, which may have impacted the results of clinical trials, e.g., IMvigor211 [4•]. It is very challenging to discern the predictive vs. prognostic role from a single-arm phase II trial; randomized trials have a better chance to assess that. Despite the numerous challenges outlined, the efforts to develop targeted therapies in mUC are robust. There are numerous ongoing clinical trials and translational studies, exploring several candidates as single agents, while combination strategies are also increasing [23]. Novel genomic sequencing and bioinformatics assays, along with further delving into tumor biology nuances, can provide a fertile ground for future endeavors.

Conclusions

Targeted therapies have not been added to the important backbone of platinum-based chemotherapy or anti-PD-(L)1 agents in mUC. At the modern era of precision therapy, new treatment strategies are very promising but need to overcome challenges related to the very complex tumor biology, molecular heterogeneity, pathway redundancy, clonal evolution, and genomic instability. Certain strategies, e.g., FGFR inhibition and ADC therapy, have provided very exciting findings resulting in breakthrough designations of two agents by FDA. Very careful development of combinations without overlapping toxicities is an important priority. Nevertheless, as supported by the several ongoing trials summarized in Table 1 (not an exhaustive list), there are opportunities to validate promising predictive biomarkers and treatment targets with potential clinical utility. Optimized biomarker-based patient selection, especially via NGS, and expansion of efficacious and safe agents may transform the management of selected patients with mUC.

Compliance with Ethical Standards

Conflict of Interest

Petros Grivas has received research funding (paid to Cleveland Clinic Foundation for conduction of clinical trials) from Genentech, Bayer, Merck & Co., Mirati Therapeutics, OncoGenex Pharmaceuticals, Pfizer, and AstraZeneca; has received research funding (paid to the University of Washington for conduction of clinical trials) from Pfizer, Clovis Oncology, Bavarian Nordic, and Immunomedics; has received compensation from Genentech, Dendreon, Bayer, Merck & Co., Pfizer, Bristol-Myers Squibb, Exelixis, AstraZeneca, Biocept, Clovis Oncology, EMD Serono, Seattle

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Human and Animal Rights and Informed Consent
This article does not contain any studies with human or animal subjects performed by any of the authors.

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