Hui *et al. J Cancer Metastasis Treat* 2022;8:34
DOI: 10.20517/2394-4722.2022.04

Journal of Cancer Metastasis and Treatment

Review

Open Access

Determinants of prognosis in metastatic urothelial carcinoma: a review of the literature

Gavin Hui[1], Ali Raza Khaki[2], Petros Grivas[3]

[1]Department of Medicine, Stanford University, Stanford, CA 94304, USA.
[2]Division of Oncology, Department of Medicine, Stanford University, Stanford, CA 94304, USA.
[3]Division of Medical Oncology, Department of Medicine, University of Washington, Fred Hutchinson Cancer Center, Seattle, WA 98109, USA.

Correspondence to: Prof. Petros Grivas, Division of Medical Oncology, Department of Medicine, University of Washington, Fred Hutchinson Cancer Center, 1144 Eastlake Ave E, Mailstop, LG-465, Seattle, WA 98109, USA. E-mail: pgrivas@uw.edu

How to cite this article: Hui G, Khaki AR, Grivas P. Determinants of prognosis in metastatic urothelial carcinoma: a review of the literature. *J Cancer Metastasis Treat* 2022;8:34. https://dx.doi.org/10.20517/2394-4722.2022.04

Received: 8 Jan 2022 **First Decision:** 2 Apr 2022 **Revised:** 14 Jul 2022 **Accepted:** 28 Jul 2022 **Published:** 22 Aug 2022

Academic Editors: Renty B. Franklin, Aristotle Bamias **Copy Editor:** Fangling Lan **Production Editor:** Fangling Lan

Abstract

The treatments for metastatic urothelial carcinoma (mUC) have advanced substantially since 2016. Prognostic tools have been used to inform clinical trial designs and treatment decisions. Historically, prognostic tools were developed for mUC based on older clinical trials involving cytotoxic chemotherapy. As novel therapies emerged, there are studies investigating prognostic factors in the era of immune checkpoint inhibitors (ICI), antibody-drug conjugates, and targeted therapies. This review aims to highlight prognostic factors in mUC and their potential in clinical decision-making and research. In the setting of chemotherapy, patient performance status, site of metastatic burden, and specific laboratory findings were found to have prognostic value in mUC. In the era of ICI, newer models identified variables such as neutrophil to lymphocyte ratio, platelet count, and lactate dehydrogenase to also have potential prognostic value. In addition to clinical biomarkers, molecular biomarkers, such as PD-L1 assay and fibroblast growth factor receptor 2 and 3 genomic testings, may have promising prognostic and predictive implications. Current methods of identifying clinical and molecular prognostic factors involve clinician insight. As large complex datasets emerge, machine learning and artificial intelligence may help data analysis and detect important prognostic features. With careful validation, such machine learning-based strategies may help create more robust prognostic and/or predictive models in the future.

Keywords: Bladder cancer, biomarkers, health outcomes, nomogram, prediction, prognosis, urothelial carcinoma, urinary tract cancers

INTRODUCTION

Urothelial carcinoma is common cancer in the United States (US) and around the world with an estimated 83,730 new cases in the US in 2021[1]. However, advances in therapy over the last few years have changed management and improved outcomes. In particular, immune checkpoint inhibitors (ICI) targeting programmed cell death protein1 (PD-1)/programmed death-ligand 1 (PD-L1) pathways, targeted therapy for FGFR2 and FGFR3 susceptible alterations and antibody-drug conjugates, enfortumab vedotin (EV) and sacituzumab govitecan (SG), have been incorporated into treatment algorithms, mainly for metastatic urothelial carcinoma (mUC).

As new therapies are approved, the use of prognostic tools can help inform clinical trial designs, patient stratification, and possibly treatment decisions. Originally, clinical prognostic tools were developed for mUC based on data from patients treated with cytotoxic chemotherapy in older clinical trials. More recently, as novel therapies have emerged, there have been several studies investigating prognostic factors in the contemporary era[2,3]. There has also been extensive work on numerous molecular biomarkers with potential prognostic and predictive implications. This (non-exhaustive) review aims to discuss examples of established and emerging prognostic factors in mUC and the potential implications in decision-making and clinical research.

BIOMARKERS, PREDICTION AND PROGNOSTICATION

Biomarkers can be derived from clinical characteristics or molecular features and can have prognostic and/or predictive properties. Prognostic biomarkers identify patients more likely to have a specific outcome [e.g., shorter or longer overall survival (OS)], regardless of therapy. Conversely, predictive biomarkers associate a particular patient benefit with a specific therapy. A number of prognostic factors may have predictive value but need to be validated prospectively to confirm clinical utility (impact on outcomes) before incorporation into routine practice[4].

Numerous risk scores aiming to assist in prognostication and/or prediction have been developed, including for patients with urothelial carcinoma. In utilizing risk algorithms, the tool's performance is tied to its ability to determine high risk from low risk in patients (biological relevance or clinical validity). Risk scores can be evaluated based on properties of calibration and discrimination. Calibration describes how well-observed outcomes align with predicted outcomes and discrimination is the ability of a model to separate individuals who will experience an event from those who will not. Calibration thus is a measure of internal and external validity, whereas discrimination can measure risk classification/stratification.

The Harrell's C index[5], a measure of the area under the receiver operator characteristic curve, is frequently used to assess how effective a tool is at discrimination. The C index ranges between 0.5 and 1.0. The higher the value, the better the model is at discrimination. A value of 0.5 indicates a very poor model, with risk score predictions similar to a coin flip in determining the outcome of interest. Conversely, a score of 1.0 would suggest perfect discrimination between those with and without the event of interest[6]. The C index ultimately is a reflection of the risk tool as well as the population in which it is deployed, and thus high-quality risk prognostication with broad applicability requires external assessment and validation.

PROGNOSTIC FACTORS

Dating back to the older cytotoxic chemotherapy clinical trials including patients with mUC, multiple clinical characteristics have been identified to have a prognostic role and have laid the foundation for subsequent prognostic risk scores. These characteristics can broadly be classified as patient fitness/wellness, treatment-associated characteristics, metastatic sites/cancer burden, and laboratory studies.

First, in terms of patient fitness, performance status (PS) is a widely used measure of patient fitness that is typically measured with one of two prominent tools - Eastern Cooperative Oncology Group Performance Status (ECOG-PS)[7] scale and Karnofsky performance status[8] (KPS). These two tools aim to provide a more objective assessment of a patient's fitness and performance with respect to activities of daily living (ADLs). Both higher ECOG-PS and lower KPS have been associated with shortened OS for patients with mUC. For example, an intergroup trial comparing methotrexate, vinblastine, doxorubicin, and cisplatin (M-VAC) with cisplatin monotherapy[9] in patients with mUC included 47 patients with KPS < 80 and found this to be significantly associated with shorter OS, with only two patients surviving at three years and only one patient at six years[10].

Second, the site(s) of metastatic burden is another factor associated with OS[9,11-13]. In the aforementioned intergroup trial of M-VAC *vs*. cisplatin monotherapy in mUC[9], patients with bone or liver metastases were found to have significantly worse responses to chemotherapy and shorter OS[10]. Similarly, in a cohort of patients treated with gemcitabine/cisplatin, the presence of visceral metastases was associated with significantly shorter OS[13].

Third, baseline laboratory studies have been identified as having prognostic values in mUC. For example, anemia (with different hemoglobin cut-off points) has been associated with worse outcomes. In a study, hemoglobin < 12.5 mg/dl was associated with adverse prognostic implications[13]. In subsequent studies and the risk scores discussed below, hemoglobin < 10 g/dl was identified to be a poor prognostic biomarker. Other laboratory measurements found to be associated with prognosis in studies include albumin, alkaline phosphatase, and lactate dehydrogenase[12].

Lastly, treatment characteristics, specifically time from prior chemotherapy, have been identified as having prognostic value. In a study of 570 patients on second-line chemotherapy, a shorter time from prior chemotherapy had a significant association with shorter OS[14]. As these various prognostic factors were identified, efforts have been made to consolidate and simplify them into prognostic risk scores or nomograms that can easily be applied in the clinical setting and/or for clinical trial design.

PROGNOSTIC MODELS

Prognostic models in the setting of chemotherapy

Table 1 summarizes examples of prognostic risk scores and nomograms that have been published. Different approaches to prognostic modeling have been used both in the selection of prognostic factors and the model output. In terms of output, many studies have developed ordinal prognostic risk scores assigning numerical values based on the number of risk factors present, and others have reported a nomogram, incorporating more continuous scale quantification accounting for the variable contribution of different risk factors to prognosis. In addition, some nomograms have pre-selected variables of interest and used multivariable models to estimate the relative weight of each variable while others have used some multi-step process to identify variables to include in the eventual nomogram or risk score.

Hui *et al. J Cancer Metastasis Treat* 2022;8:34 | https://dx.doi.org/10.20517/2394-4722.2022.04

Table 1. Prognostic risk scores and nomograms in metastatic urothelial carcinoma

Study	Year of publication	N (development; validation)	Population	Trial or RW	Outcome	OS (months)	Factors	C-statistic (internal; validation)
Geller *et al.*[56]	1991	92	First-line M-VAC chemotherapy	Trial	OS	n/a	Low KPS, High Alkaline phosphatase, age < 60	NR
Bajorin *et al.*[15]	1999	203	First-line M-VAC chemotherapy	Trial	OS	33 (0 RF), 13.4 (1 RF), 9.3 (2 RF)	KPS < 80, Visceral metastases	NR
Bellmunt *et al.*[12]	2002	56	First-line TCG chemotherapy	Trial	OS	32.8 (0 RF), 17 (1 RF), 9.6 (2 RF)	ECOG-PS > 0, Visceral metastases	NR
Lin *et al.*[57]	2007	79	First-line P-HDFL chemotherapy	Trial	OS	81.8 (0 RF), 13.2 (1-2 RF), 4.6 (3 RF)	KPS < 80, Visceral metastases, alkaline phosphatase > 220 U/L	NR
Bellmunt *et al.*[16]	2010	370; 151	Post-platinum therapy	Trial	OS	14.2 (0 RF), 7.3 (1 RF), 3.8 (2 RF), 1.7 (3 RF)	ECOG-PS > 0, Liver metastasis, Hgb < 10 g/dl	NR
Apolo *et al.*[58]	2013	308; 74	Cisplatin-based chemotherapy	Trial	OS	12.7	Nomogram: albumin, hemoglobin, KPS, visceral metastases	0.67; 0.63
Sonpavde *et al.*[14]	2013	570; 352	Second-line chemotherapy	Trial	OS	12.2 (0 RF), 6.7 (1 RF), 5.1 (2 RF), 3.0 (3-4 RF)	ECOG-PS > 0, Liver metastases, Hgb < 10 g/dl, Time since last chemotherapy < 3 months	NA
Galsky *et al.*[17]	2013	384; 186	Cisplatin-based chemotherapy	Trial	OS	13.8	Nomogram: ECOG-PS (0, 1, 2+), Leukocytosis (WBC > ULN), Number of sites of visceral metastases (0,1, 2-3), Site of primary tumor (Bladder *vs.* other), Lymph node metastases (yes *vs.* no)	0.63; 0.63
Galsky *et al.*[18]	2014	375; 182	Cisplatin-based chemotherapy (post-treatment)	Trial	OS	10.65	Nomogram: ECOG-PS (0, 1, 2+), Leukocytosis (WBC > ULN), Number of sites of visceral metastases (0,1, 2 or 3), Response to chemotherapy (CR, PR, SD, PD)	0.68; 0.67
Pond *et al.*[59]	2014	570	Second-line chemotherapy	Trial	6-month PFS	n/a	Nomogram: ECOG-PS (0, 1+), Liver metastases, Hgb < 10 g/dl, TFPC (< 3 months, 3-6 months, > 6 months)	0.62
Salah *et al.*[60]	2016	193; 44	Second-line chemotherapy	RW	OS	13.3 (0 RF), 8.1 (1-2 RF), 3.3 (3 RF)	ECOG-PS > 0, non-lymph node-only metastases, Hgb < 10 g/dl,	NR
Sonpavde *et al.*[61]	2016	444; 167	Salvage chemotherapy	Trial	OS	10.6 (0-1 RF), 10.0 (2 RF), 7.0 (3+ RF)	ECOG-PS > 0, Liver metastases, Hgb < 10g/dl, TFPC < 3 months, Albumin < LLN	0.64; 0.65
Necchi *et al.*[62]	2017	687; 333	First-line platinum-based chemotherapy	RW	OS	n/a	Nomogram: WBC, ECOG-PS, BMI, Lung/Liver/Bone metastases, ethnicity, perioperative chemotherapy	0.67; 0.66
Nassar *et al.*[22]	2019	62	ICI (any line)	RW	No clinical benefit	n/a	Visceral metastases, NLR ≥ 5, single-nucleotide variant count < 10	0.90
Nelson *et al.*[63]	2020	270	Any systemic therapy	RW	OS	6.1	Early bone metastases	NR
Sonpavde	2020	405; 242 and 198	Post-platinum atezolizumab	Trial	OS	n/a	Liver metastases, ECOG-PS > 0, NLR[a], platelet count[a],	0.69; 0.67

et al.[2]			(training), avelumab and durvalumab (validation)				lactate dehydrogenase[a]	(avelumab) and 0.77 (durvalumab)
Khaki *et al.*[3]	2020	357	First-line ICI	RW	OS	23 (0 RS), 10 (1 RS), 5 (2 RS)	ECOG-PS > 1, Liver metastases, Albumin < LLN, NLR > 5	0.68
Kobayashi *et al.*[19]	2021	463; 292	Post-platinum pembrolizumab	RW	OS	6.8 (int risk) 2.3 (high risk)	ECOG-PS: 0, 1, 2+; Metastatic site: Lymph node only, other organs, liver; Hgb < 11 g/dl; NLR ≥ 3	0.73; 0.75
Ruiz-Bañobre *et al.*[20]	2021	119	ICI (any line)	RW	OS	7.8	ECOG-PS ≥ 2, Liver metastases, peritoneal metastasis, albumin levels < 3.5 g/dl, proton-pump inhibitor use	NR

[a]log-transformed; ECOG: Eastern cooperative oncology group; ICI: immune checkpoint inhibitors; LLN: lower limit of normal; M-VAC: methotrexate, vinblastine, doxorubicin and cisplatin; NLR: neutrophil to lymphocyte ratio; NR: not reported; OS: overall survival; PFS: progression-free survival; P-HDFL: cisplatin, high-dose 5-fluorouracil/leucovorin; PS: Performance status; RF: Risk factor; RS: Risk score; RW: Real-world; TCG: paclitaxel, cisplatin, gemcitabine; TFPC: Time from previous chemotherapy.

Two prominent prognostic risk scores in mUC that have been widely used in clinical practice and have been incorporated into clinical trial reporting are the Bajorin and Bellmunt models. The Bajorin risk factors were identified over 20 years ago in a retrospective cohort of about 200 patients with unresectable or metastatic UC treated with M-VAC chemotherapy regimen in a clinical trial[15]. The authors found that a low KPS score (< 80) and the presence of visceral metastases were associated with shorter OS. The Bellmunt score was developed in a population of 370 patients who progressed after first-line platinum-based chemotherapy and were enrolled in a clinical trial evaluating vinflunine. The negative prognostic factors identified included hemoglobin < 10 g/dl, liver metastasis, and ECOG-PS > 0[16]. These risk scores suggested that the presence of visceral metastases, more specifically liver metastases, and poor PS portended a much worse prognosis. Notably, both scores were developed in cohorts receiving cytotoxic chemotherapy (first and second line, respectively) and while they have been used in modern clinical trial reporting, more recent contemporary models have also been developed.

In addition to the Bajorin and Bellmunt scores, other prognostic models prior to ICI were similarly developed in patients receiving chemotherapy in clinical trials. Many of the risk factors identified were similar, with a measure of performance status and often either visceral or, specifically, liver metastases. A study by Sonpavde *et al.* attempted to build on the Bellmunt score for second-line chemotherapy and identified an additional prognostic value of the time interval since the last chemotherapy (< 3 *vs.* ≥ 3 months)[14]. A series of studies by Galsky *et al.* developed nomograms for OS for patients treated with cisplatin-based first-line chemotherapy with a nomogram for patients prior to the start of chemotherapy[17] and another after chemotherapy[18]. In both studies, ECOG-PS, lymphocytosis, and the number of visceral metastases sites were included in the risk model. In the pre-treatment study, the site of the primary tumor (bladder *vs.* other) and the presence of lymph node metastases were also included in the nomogram. In the post-treatment nomogram, response to chemotherapy (complete response, partial response, stable disease, or progression) also added prognostic value.

Prognostic models in the setting of ICI

The approval of ICI for patients with locally advanced, unresectable or metastatic UC has revolutionized the care and therapeutic landscape for this population. These new therapies and mechanisms of action with less toxicity (compared to cytotoxic chemotherapy) and the potential for durable responses raised the question of whether previously developed prognostic models were still applicable. While data from trials suggested that the Bellmunt factors still maintain prognostic relevance, more contemporary models, using both clinical trial-based and "real-world setting" cohorts, have been developed in patients with mUC treated with ICI [Table 1]. Such models may be more relevant in the context of ICI therapy regarding prognostication and clinical trial design, e.g., stratification factors.

A model developed by Sonpavde *et al.* was built for patients with mUC treated with ICI after platinum-based chemotherapy[2]. This model included patients from three clinical trials of PD-L1 inhibitors (atezolizumab, avelumab, durvalumab). The derivation dataset included two phase I/II trials of atezolizumab (n = 405) and identified five statistically significant clinical and laboratory prognostic factors [higher ECOG-PS, higher neutrophil to lymphocyte ratio (NLR), higher platelet count, higher lactate dehydrogenase, and presence of liver metastasis, all associated with shorter survival][2]. The model was then validated using datasets from clinical trials of avelumab (n = 242) and durvalumab (n = 198). The C-statistics were 0.69, 0.67, and 0.77 in the atezolizumab, avelumab and durvalumab datasets, respectively.

In the first-line setting, a new risk score was developed by Khaki *et al.* using real-world data from a large multi-institution database across the US and Europe[3]. This data was from 24 institutions and five countries and included 357 patients. This model identified ECOG-PS > 1, liver metastases, albumin < lower limit of normal, and NLR > 5 as poor prognostic features with a C-statistic of 0.68. A limitation of this risk score is the absence of an external validation cohort. Another real-world prognostic model was developed by Kobayashi *et al.* in Japan and identified ECOG-PS (0, 1, 2+), metastatic site (lymph node only, other organs, liver), hemoglobin < 11 g/dL, and NLR ≥ 3 as significant prognostic factors with C-statistics of 0.73 and 0.75[19]. Notably, common features of these models developed in the setting of ICI therapy are the persistent prognostic value of ECOG-PS and metastatic sites (especially liver), and a recently shared factor was the high NLR, despite different cut-off levels.

Another study with 119 patients with mUC under anti-PD-(L)1 therapy identified a three-risk category prognostic model including ECOG-PS, PPI use, albumin level, presence of liver metastases, and presence of peritoneal metastases[20]. Patients with these variables were associated with a higher risk of death (HR = 3, 95%CI: 1.97-4.56). In this study, the presence of peritoneal metastases was an independent prognostic factor for shorter OS. Although it is unclear why PPI use would be associated with worse outcomes, another study comparing atezolizumab versus chemotherapy also found that PPI use was also associated with shorter OS and PFS with atezolizumab, but not chemotherapy[21].

While contemporary clinical prognostic models have updated several factors based on more recent therapies, they have still been limited by only moderate discrimination with C-statistic around 0.7. One potential approach to improve the prognostic characteristics would be to integrate molecular biomarkers with clinical data. This was done by Nassar *et al.* in a small single-institution study (N = 62), developing a model with a C-statistic of 0.9[22]. However, there was no external validation. In this model, they identified NLR < 5, single-nucleotide variant count > 9, and lack of visceral metastases to be associated with the benefits of ICI. As molecular testing (e.g., next-generation sequencing) becomes more broadly used in clinical practice, incorporation of molecular information into future modeling efforts may improve prognostication. However, healthcare disparities may impact access and use of molecular testing across

populations, and this needs to be addressed in a systematic standardized way.

MOLECULAR BIOMARKERS

Identifying biomarkers with clinical utility depends on several steps. A robust molecular biomarker needs to have excellent pre-analytics, analytical validity, clinical validity, and clinical utility. Analytical validity is a measure of reproducibility and accuracy of a test/assay. The second step is demonstrating clinical validity (biological relevance). A biomarker needs to be clinically meaningful and able to identify the disease (or a certain outcome) from others. Lastly, clinical utility is a broader measurement of the test's usefulness in patient care. This takes into account the risks and benefits of the tests and how they can be used to inform clinical outcomes[23,24]. There are blood-, urine-, and stool-based putative molecular biomarkers. In mUC, blood-based molecular biomarkers are more commonly studied, while urine-based biomarkers may be promising in earlier stages of UC. Examples of blood-based molecular biomarkers include circulating tumor cells (CTCs), circulating tumor DNA (ctDNA), cell-free micro RNA, or exosomes[25].

CTCs are rare tumor cells that may be detected in the blood. The prognostic/predictive role of CTCs is still being defined but very early studies estimated the prevalence of CTCs to be 50% (7/14 patients) among those with mUC *vs*. only 17% (5/30) with clinically localized UC[26]. CTCs have also been shown to be associated with increased progression to metastatic disease after radical cystectomy[27] and early changes in CTCs while on treatment for mUC may impact PFS and OS[28].

Another very promising biomarker is circulating tumor DNA (ctDNA). ctDNA also has potential implications in therapy monitoring, determining minimal residual disease (MRD), and identifying clonal evolution and new genomic alterations, as potential resistance mechanisms[29]. For example, in a study of 51 patients with advanced bladder cancer, ctDNA provided information about somatic alterations, with 95% of patients found to have mutations in TP53, Rb1, or MDM2 and 20% with ERBB2 amplification[30]. In a separate study of 68 patients with advanced bladder cancer, ctDNA was shown to be helpful in risk stratification and detecting early recurrence[31]. Other studies have also suggested ctDNA can identify disease progression[32], can inform prognosis[33] and identify potential predictive biomarkers (e.g., FGFR2/3 alterations)[34,35]. Recent data from an exploratory analysis from the IMvigor010 trial of adjuvant atezolizumab *vs*. observation suggested that the use of ctDNA after radical cystectomy may be not only a prognostic but also a predictive biomarker. While the intention to treat the population in that trial did not show improved survival with adjuvant atezolizumab *vs*. observation, patients considered ctDNA-positive after radical surgery had shorter DFS and OS, while atezolizumab improved survival only in this subgroup[29]. Clinical trials using ctDNA to identify MRD and guide subsequent therapy are underway in two studies: IMvigor011 and Tombola. More specifically, IMvigor011 is using ctDNA to identify patients with MRD after radical surgery, randomizing patients with +ctDNA to either atezolizumab or placebo, accordingly. The Tombola trial is using ctDNA monitoring to guide the initiation of atezolizumab after radical surgery.

MicroRNAs have also been studied as a potential biomarker of prognosis. High expression of microRNA 21, among other biomarkers, has been associated with shorter OS[36]. Blood-based measurements of cell-free microRNA may become an important surveillance tool in mUC in the future, upon further validation. These studies offer a glimpse of the future in the attempt of personalized and precision oncology. However, molecular biomarkers have not been validated in large clinical trials. So far, these studies have demonstrated the feasibility of incorporating biomarker evaluation in future clinical trials.

In mUC, PD-L1 assays and FGFR 2 or 3 susceptible genomic alterations (activating mutation, fusion, rearrangement) are the only biomarkers with current possible implications in clinical practice. Multiple PD-L1 assays have been investigated with immune checkpoint inhibitor development. However, only the Ventana SP142 assay is currently associated with an FDA treatment indication for mUC. Treatment-naïve, cisplatin-ineligible, but carboplatin-eligible, patients with ≥ 5% tumor-infiltrating immune cells can be eligible for front-line atezolizumab monotherapy based on this biomarker[37,38]. PD-L1 assays have otherwise been limited by lack of reproducibility between assays, variable antibodies used, different cutoffs, cells measured, and scoring algorithms, limiting further development[39].

FGFR2 and FGFR3 testing has become an important biomarker because the FGFR inhibitor, erdafitinib, demonstrated a high response rate (~40%) in patients with platinum-refractory mUC. This resulted in accelerated FDA approval of this agent, based on the single-arm phase II BLC2001trial[40]. FGFRs (fibroblast growth factor receptors) are tyrosine kinase receptors involved in oncogenesis. FGFR gene fusions can be detected with diagnostic RT-PCR, which is a reverse transcriptase-linked polymerase chain reaction. More recently, next-generation sequencing (NGS) assays, e.g., using DNA and RNA-based approach, have also improved the detection of FGFR gene fusions[41]. In the BLC2001 trial leading to the accelerated FDA approval of erdafitinib, patients had FGFR2 or FGFR3 activating mutation or fusion assessed by RT-PCR assay (Qiagen Therascreen), which received approval as a companion diagnostic[40,42].

Outside of FDA approval, there are several other emerging biomarkers related to ICI treatment of mUC. Many putative ICI biomarkers have potential prognostic implications, provided that they can be validated in large studies. This literature review on UC immunotherapy identified host and tumor-specific parameters aimed to improve understanding of patients' responses to immunotherapy[43,44]. Other specific emerging biomarkers include tumor mutational burden, microsatellite instability, and DNA damage response. Tumor mutational burden (TMB) is the estimated measurement of mutations per Megabase and it has been associated with longer OS in mUC in a few studies[38,43]. It has also been suggested as a tumor-agnostic predictive biomarker for ICI (based on pembrolizumab FDA approval across pre-treated solid tumors). Similarly, microsatellite instability-high has also been suggested as a predictive biomarker of ICI response and pembrolizumab also has tumor-agnostic FDA approval for this indication. Microsatellite instability-high has been estimated to occur in ~1%-2% of urothelial carcinoma and has been associated with durable responses to ICI in small retrospective studies[45,46]. DNA damage response (DDR) genes and their alterations make up an additional putative biomarker. The presence of DDR alterations was found to be associated with higher response rates in 60 patients with mUC treated with anti-PD-1 or anti-PDL1[47]. Ultimately, rather than a single prognostic or predictive biomarker with ICI therapy, the best biomarker may be composed of a combination of molecular biomarkers and clinical information. This was suggested with the previously discussed prognostic risk score developed by Nassar *et al.*[22]and has also been suggested in some exploratory studies from clinical trials[48,49].

In addition to biomarkers related to ICI treatment and erdafitinib, there is additional work to identify potential biomarkers for antibody-drug conjugates, such as enfortumab vedotin and sacituzumab govitecan. Immunohistochemistry or molecular assays to identify the target antigen expression (Nectin-4 and Trop-2, respectively) could have prognostic and/or predictive utility, though neither drug was approved with a requisite companion diagnostic. Notably, all patients in the EV-201 study leading to accelerated approval of enfortumab vedotin had detectable Nectin-4 expression, though the ORR from the trial was only 44%, which may suggest the limited predictive utility of expression assessment. However, other studies have shown more heterogeneity in Nectin-4 expression[50,51], with *in vitro* data suggesting Nectin-4 expression is necessary for enfortumab vedotin clinical activity[50]. Further work to identify cut points for degree of

protein expression based on H-score may still have predictive potential. In a retrospective hypothesis-generating study in a cohort of patients with advanced UC, the presence of TP53 and the absence of CDKN2A and CDKN2B alterations were associated with favorable responses with EV[52]. The UNITE study investigating the real-world effectiveness of enfortumab vedotin has also identified subgroups, including those with variant histology, presence of liver metastasis, FGFR3 alterations, and those with medical comorbidities (e.g., GFR < 30 ml/min) usually excluded from clinical trials, that had similar clinical outcomes with EV therapy[53]. Trop-2 has also been shown to have high expression across bladder cancer subtypes with the exception of neuroendocrine subtype[54], and response to sacituzumab govitecan is seen across expression levels (though higher with higher expression)[55]. However, the ORR with sacituzumab govitecan is only ~30%, which suggests that further work to identify a predictive biomarker could be helpful. Moreover, other clinical or molecular features (aside from antigen expression) may also have prognostic and/or predictive utility related to ADC therapy.

Importantly, as molecular biomarkers are developed, it is essential to include them in clinical trials. However, for this to be done successfully, there will ideally be a need for standardization of biospecimen acquisition and biomarker analysis. Standardizing assays, instruments, tubes, time points of collection, processing, and storage conditions can help further evaluate the clinical validity and utility of putative biomarkers.

FUTURE DIRECTIONS

As the therapeutic landscape of mUC evolves with newer therapeutic agents, clinical prognostication and prediction become essential to inform the optimal treatment sequence for each individual patient.

As electronic health records become more robust and standardized, clinical prognostication has the potential to improve with aid from tools using machine learning and artificial intelligence methods to better process and extract insight from existing large datasets. While most clinical prognostication discussed to date has relied on clinician identification of relevant prognostic factors, machine learning methods have the potential to detect important features from complex datasets that may not be readily identified by a clinician. The application of these methods may further improve clinical prognostication and may even identify predictive biomarkers related to specific therapies. However, these new models will still need to be properly validated in prospective studies before they can be used in the clinical setting.

Further, given the molecular/targeted nature of novel agents in mUC, there is also tremendous potential for further predictive biomarker development. In this review, we have highlighted examples of potentially promising molecular biomarkers, including CTCs, ctDNA, NGS, IHC, and gene expression assays, among others with prognostic and/or predictive potential. Further work to identify the optimal use and standardization of assays can assist in the optimal validation, clinical implementation, and adoption. In addition, efforts should be made to appropriately structure and digitize relevant molecular information so it is analytically accessible for machine learning methods to inform biomarker development and potentially incorporate both clinical and molecular information in future prognostic and predictive tools.

CONCLUSION

Our review has identified recent studies that revealed clinical prognostic factors in mUC. While most of these factors were identified in the setting of chemotherapy, they also seem relevant in patients treated with ICI. The two most common clinical prognostic factors have been the presence of visceral/liver metastases and poor performance status. NLR and albumin levels appear to likely be significant factors in those treated with ICI.

While there are several clinical prognostic factors, the strive to identify molecular biomarkers is also essential. Nassar *et al.*'s model had one of the highest C-statistics regarding prognosis in mUC, suggesting that incorporating other variables besides clinical factors may increase prognostic value[22].

Patient selection based on biomarker analysis would be helpful if they can predict favorable outcomes for specific therapies. ctDNA also has the potential to improve treatment monitoring and even guide the initiation of therapies. TMB, ctDNA, and PD-L1 staining assays may become important molecular factors in future prognostic models.

Future studies should include biomarkers of interest in the ICI era, including but not limited to TMB, T effector and regulatory cells, PD-L1 protein expression, EMT and tumor stroma, TGF-β, FGFR2 and FGFR3 alterations, among others[43]. Moreover, PDL1 immunostaining and FGFR2/3 susceptible genomic alterations can be used to help select specific therapies[42].

As healthcare becomes more "digitalized", there will be a lot of patient-generated data from electronic health records that can be analyzed with AI and ML to create more robust prognostic and possibly predictive models that can be properly validated in the future.

DECLARATIONS

Authors' contributions

Wrote initial draft: Hui, G
Made substantial contributions to conception and design of the study, critical review of the manuscript: Khaki AR, Grivas P

Availability of data and materials

Data available in the resources listed in references.

Financial support and sponsorship

None.

Conflicts of interest

Petros Grivas: consulting or advisory role: 4D Pharma PLC, Astellas Pharma, AstraZeneca, Boston Gene, Bristol Myers Squibb, Dyania Health, EMD Serono, Exelixis, Fresenius Kabi, Genentech, Gilead Sciences, Guardant Health, Infinity Pharmaceuticals, Janssen, Lucence Health, Merck, Mirati Therapeutics, Pfizer, PureTech, QED Therapeutics, Regeneron Pharmaceuticals, Roche, Seattle Genetics, Silverback Therapeutics, UroGen; institutional research funding: Bavarian Nordic, Bristol Myers Squibb, Clovis Oncology, Debiopharm Group, EMD Serono, G1 Therapeutics, Gilead Sciences, GlaxoSmithKline, Merck, Mirati Therapeutics, Pfizer, QED Therapeutics. ARK: Stock ownership in Merck and Sanofi; research collaboration with Tempus and Natera, honoraria from MJH Life Sciences.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

REFERENCES

1. American Cancer Society. Key statistics for bladder cancer. Available from: https:// www.cancer.org/cancer/bladder-cancer/about/key-statistics.html [Last accessed on 3 Aug 2022].
2. Sonpavde G, Manitz J, Gao C, et al. Five-factor prognostic model for survival of post-platinum patients with metastatic urothelial carcinoma receiving PD-L1 inhibitors. *J Urol* 2020;204:1173-9. DOI PubMed PMC
3. Khaki AR, Li A, Diamantopoulos LN, et al. A new prognostic model in patients with advanced urothelial carcinoma treated with first-line immune checkpoint inhibitors. *Eur Urol Oncol* 2021;4:464-72. DOI PubMed PMC
4. Khaki AR, Gadi VK, Prasad V. Clinical risk during the evaluation of genomic risk for hormone-sensitive breast cancer: ignoring valuable data. *J Natl Compr Canc Netw* 2019;17:1456-8. DOI PubMed
5. Harrell FE. Evaluating the yield of medical tests. *JAMA* 1982;247:2543. PubMed
6. Harrell FE, Lee KL, Mark DB. Multivariable prognostic models: issues in developing models, evaluating assumptions and adequacy, and measuring and reducing errors. *Statist Med* 1996;15:361-87. DOI PubMed
7. Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *J Clin Oncol* 1982;5:649-656. PubMed
8. Karnofsky performance status - an overview|sciencedirect topics. Available from: https://www.sciencedirect.com/topics/medicine-and-dentistry/karnofsky-performance-status [Last accessed on 3 Aug 2022].
9. Loehrer PJ Sr, Einhorn LH, Elson PJ, et al. A randomized comparison of cisplatin alone or in combination with methotrexate, vinblastine, and doxorubicin in patients with metastatic urothelial carcinoma: a cooperative group study. *J Clin Oncol* 1992;10:1066-73. DOI PubMed
10. Saxman SB, Propert KJ, Einhorn LH, et al. Long-term follow-up of a phase III intergroup study of cisplatin alone or in combination with methotrexate, vinblastine, and doxorubicin in patients with metastatic urothelial carcinoma: a cooperative group study. *J Clin Oncol* 1997;15:2564-9. DOI PubMed
11. von der Maase H, Sengelov L, Roberts JT, et al. Long-term survival results of a randomized trial comparing gemcitabine plus cisplatin, with methotrexate, vinblastine, doxorubicin, plus cisplatin in patients with bladder cancer. *JCO* 2005;23:4602-8. DOI PubMed
12. Bellmunt J, Albanell J, Paz-Ares L, et al. Pretreatment prognostic factors for survival in patients with advanced urothelial tumors treated in a phase I/II trial with paclitaxel, cisplatin, and gemcitabine. *Cancer* 2002;95:751-7. DOI PubMed
13. Stadler WM, Hayden A, von der Maase H, et al. Long-term survival in phase II trials of gemcitabine plus cisplatin for advanced transitional cell cancer. *Urol Oncol Semin Origin Investigat* 2002;7:153-7. DOI PubMed
14. Sonpavde G, Pond GR, Fougeray R, et al. Time from prior chemotherapy enhances prognostic risk grouping in the second-line setting of advanced urothelial carcinoma: a retrospective analysis of pooled, prospective phase 2 trials. *Eur Urol* 2013;63:717-23. DOI PubMed PMC
15. Bajorin DF, Dodd PM, Mazumdar M, et al. Long-term survival in metastatic transitional-cell carcinoma and prognostic factors predicting outcome of therapy. *J Clin Oncol* 1999;17:3173-81. DOI PubMed
16. Bellmunt J, Choueiri TK, Fougeray R, et al. Prognostic factors in patients with advanced transitional cell carcinoma of the urothelial tract experiencing treatment failure with platinum-containing regimens. *J Clin Oncol* 2010;28:1850-5. DOI PubMed
17. Galsky MD, Moshier E, Krege S, et al. Nomogram for predicting survival in patients with unresectable and/or metastatic urothelial cancer who are treated with cisplatin-based chemotherapy. *Cancer* 2013;119:3012-9. DOI PubMed
18. Galsky MD, Moshier E, Krege S, et al. Posttreatment prognostic nomogram for patients with metastatic urothelial cancer completing first-line cisplatin-based chemotherapy. *Urol Oncol* 2014;32:48.e1-8. DOI PubMed
19. Kobayashi T, Ito K, Kojima T, et al. Risk stratification for the prognosis of patients with chemoresistant urothelial cancer treated with pembrolizumab. *Cancer Sci* 2021;112:760-73. DOI PubMed PMC
20. Ruiz-Bañobre J, Molina-Díaz A, Fernández-Calvo O, et al. Rethinking prognostic factors in locally advanced or metastatic urothelial carcinoma in the immune checkpoint blockade era: a multicenter retrospective study. *ESMO Open* 2021;6:100090. DOI PubMed PMC
21. Hopkins AM, Kichenadasse G, Karapetis CS, Rowland A, Sorich MJ. Concomitant proton pump inhibitor use and survival in urothelial carcinoma treated with atezolizumab. *Clin Cancer Res* 2020;26:5487-93. DOI PubMed
22. Nassar AH, Mouw KW, Jegede O, et al. A model combining clinical and genomic factors to predict response to PD-1/PD-L1 blockade in advanced urothelial carcinoma. *Br J Cancer* 2020;122:555-63. DOI PubMed PMC
23. Teutsch SM, Bradley LA, Palomaki GE, et al. The evaluation of genomic applications in practice and prevention (EGAPP) initiative: methods of the EGAPP Working Group. *Genet Med* 2009;11:3-14. DOI PubMed PMC
24. Hayes DF. Biomarker validation and testing. *Mol Oncol* 2015;9:960-6. DOI PubMed PMC
25. Neumann MHD, Bender S, Krahn T, Schlange T. ctDNA and CTCs in liquid biopsy - current status and where we need to progress. *Comput Struct Biotechnol J* 2018;16:190-5. DOI PubMed PMC
26. Flaig TW, Wilson S, van Bokhoven A, et al. Detection of circulating tumor cells in metastatic and clinically localized urothelial carcinoma. *Urology* 2011;78:863-7. DOI PubMed PMC

27. Abrahamsson J, Aaltonen K, Engilbertsson H, et al. Circulating tumor cells in patients with advanced urothelial carcinoma of the bladder: Association with tumor stage, lymph node metastases, FDG-PET findings, and survival. *Urol Oncol* 2017;35:606.e9-606.e16. DOI PubMed
28. Fina E, Necchi A, Giannatempo P, et al. Clinical significance of early changes in circulating tumor cells from patients receiving first-line cisplatin-based chemotherapy for metastatic urothelial carcinoma. *Bladder Cancer* 2016;2:395-403. DOI PubMed PMC
29. Powles T, Assaf ZJ, Davarpanah N, et al. ctDNA guiding adjuvant immunotherapy in urothelial carcinoma. *Nature* 2021;595:432-7. DOI PubMed
30. Vandekerkhove G, Todenhöfer T, Annala M, et al. Circulating tumor DNA reveals clinically actionable somatic genome of metastatic bladder cancer. *Clin Cancer Res* 2017;23:6487-97. DOI PubMed
31. Christensen E, Birkenkamp-Demtröder K, Sethi H, et al. Early detection of metastatic relapse and monitoring of therapeutic efficacy by ultra-deep sequencing of plasma cell-free dna in patients with urothelial bladder carcinoma. *J Clin Oncol* 2019;37:1547-57. DOI PubMed
32. Shohdy KS, Villamar DM, Cao Y, et al. Serial ctDNA analysis predicts clinical progression in patients with advanced urothelial carcinoma. *Br J Cancer* 2022;126:430-9. DOI PubMed PMC
33. Grivas P, Lalani AA, Pond GR, et al. Circulating tumor DNA alterations in advanced urothelial carcinoma and association with clinical outcomes: a pilot study. *Eur Urol Oncol* 2020;3:695-9. DOI PubMed
34. Agarwal N, Pal SK, Hahn AW, et al. Characterization of metastatic urothelial carcinoma via comprehensive genomic profiling of circulating tumor DNA. *Cancer* 2018;124:2115-24. DOI PubMed PMC
35. Grivas P, Kiedrowski LA, Sonpavde GP, et al. Spectrum of FGFR2/3 alterations in cell-free DNA of patients with advanced urothelial carcinoma. *BLC* 2021;7:143-8. DOI
36. Xie Y, Ma X, Chen L, et al. MicroRNAs with prognostic significance in bladder cancer: a systematic review and meta-analysis. *Sci Rep* 2017;7:5619. DOI PubMed PMC
37. Stenehjem DD, Tran D, Nkrumah MA, Gupta S. PD1/PDL1 inhibitors for the treatment of advanced urothelial bladder cancer. *Onco Targets Ther* 2018;11:5973-89. DOI PubMed PMC
38. Balar AV, Galsky MD, Rosenberg JE, et al. Atezolizumab as first-line treatment in cisplatin-ineligible patients with locally advanced and metastatic urothelial carcinoma: a single-arm, multicentre, phase 2 trial. *Lancet* 2017;389:67-76. DOI PubMed PMC
39. Udall M, Rizzo M, Kenny J, et al. PD-L1 diagnostic tests: a systematic literature review of scoring algorithms and test-validation metrics. *Diagn Pathol* 2018;13:12. DOI PubMed PMC
40. Loriot Y, Necchi A, Park SH, et al. Erdafitinib in locally advanced or metastatic urothelial carcinoma. *N Engl J Med* 2019;381:338-48. DOI PubMed
41. De Luca A, Esposito Abate R, Rachiglio AM, et al. FGFR fusions in cancer: from diagnostic approaches to therapeutic intervention. *Int J Mol Sci* 2020;21:6856. DOI PubMed PMC
42. Research C for DE and FDA grants accelerated approval to erdafitinib for metastatic urothelial carcinoma. Available from: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-grants-accelerated-approval-erdafitinib-metastatic-urothelial-carcinoma [Last accessed on 3 Aug 2022].
43. Dijk N, Funt SA, Blank CU, Powles T, Rosenberg JE, van der Heijden MS. The cancer immunogram as a framework for personalized immunotherapy in urothelial cancer. *Eur Urol* 2019;75:435-44. DOI PubMed PMC
44. Zahoor H, Grivas P. The cancer immunogram: a pledge for a comprehensive biomarker approach for personalized immunotherapy in urothelial cancer. *Eur Urol* 2019;75:445-7. DOI PubMed
45. Fraune C, Simon R, Hube-Magg C, et al. MMR deficiency in urothelial carcinoma of the bladder presents with temporal and spatial homogeneity throughout the tumor mass. *Urol Oncol* 2020;38:488-95. DOI PubMed
46. Sarfaty M, Teo MY, Al-ahmadie H, et al. Microsatellite instability (MSI-H) in metastatic urothelial carcinoma (mUC): a biomarker of divergent responses to systemic therapy. *JCO* 2020;38:566-566. DOI
47. Teo MY, Seier K, Ostrovnaya I, et al. Alterations in DNA damage response and repair genes as potential marker of clinical benefit from PD-1/PD-L1 blockade in advanced urothelial cancers. *J Clin Oncol* 2018;36:1685-94. DOI PubMed PMC
48. Powles T, Sridhar SS, Loriot Y, et al. Avelumab maintenance in advanced urothelial carcinoma: biomarker analysis of the phase 3 JAVELIN bladder 100 trial. *Nat Med* 2021;27:2200-11. DOI PubMed
49. Bellmunt J, de Wit R, Fradet Y, et al. Putative biomarkers of clinical benefit with pembrolizumab in advanced urothelial cancer: results from the KEYNOTE-045 and KEYNOTE-052 landmark trials. *Clin Cancer Res* 2022;28:2050-60. DOI PubMed
50. Chu CE, Sjöström M, Egusa EA, et al. Heterogeneity in. *NECTIN4* ;27:5123-30. DOI PubMed PMC
51. Hoffman-Censits JH, Lombardo KA, Parimi V, et al. Expression of nectin-4 in bladder urothelial carcinoma, in morphologic variants, and nonurothelial histotypes. *Appl Immunohistochem Mol Morphol* 2021;29:619-25. DOI PubMed PMC
52. Jindal T, Zhang L, Chou J, et al. Biomarkers predictive of response to enfortumab vedotin (EV) treatment in advanced urothelial cancer (aUC). *JCO* 2022;40:531-531. DOI
53. Koshkin VS, Henderson N, James M, et al. Efficacy of enfortumab vedotin in advanced urothelial cancer: Analysis from the Urothelial Cancer Network to Investigate Therapeutic Experiences (UNITE) study. *Cancer* 2022;128:1194-205. DOI PubMed
54. Chou J, Trepka K, Sjöström M, et al. TROP2 Expression across molecular subtypes of urothelial carcinoma and enfortumab vedotin-resistant cells. *Eur Urol Oncol* ;2022:S2588-9311(21)00215. DOI PubMed
55. Loriot Y, Balar A, Petrylak D, et al. 700P efficacy of sacituzumab govitecan (SG) by trophoblast cell surface antigen 2 (Trop-2)

expression in patients (Pts) with metastatic urothelial cancer (mUC). *Ann Oncol* 2021;32:S712-3. DOI

56. Geller NL, Sternberg CN, Penenberg D, Scher H, Yagoda A. Prognostic factors for survival of patients with advanced urothelial tumors treated with methotrexate, vinblastine, doxorubicin, and cisplatin chemotherapy. *Cancer* 1991;67:1525-31. DOI PubMed
57. Lin CC, Hsu CH, Huang CY, et al. Prognostic factors for metastatic urothelial carcinoma treated with cisplatin and 5-fluorouracil-based regimens. *Urology* 2007;69:479-84. DOI PubMed
58. Apolo AB, Ostrovnaya I, Halabi S, et al. Prognostic model for predicting survival of patients with metastatic urothelial cancer treated with cisplatin-based chemotherapy. *J Natl Cancer Inst* 2013;105:499-503. DOI PubMed PMC
59. Pond GR, Agarwal N, Bellmunt J, et al. A nomogram including baseline prognostic factors to estimate the activity of second-line therapy for advanced urothelial carcinoma. *BJU Int* 2014;113:E137-43. DOI PubMed
60. Salah S, Lee JL, Rozzi A, et al. Second-line chemotherapy for metastatic urothelial carcinoma: importance of lymph node-only metastasis as a prognostic factor and construction of a prognostic model. *Clin Genitourin Cancer* 2016;14:255-60. DOI PubMed
61. Sonpavde G, Pond GR, Rosenberg JE, et al. Improved 5-factor prognostic classification of patients receiving salvage systemic therapy for advanced urothelial carcinoma. *J Urol* 2016;195:277-82. DOI PubMed PMC
62. Necchi A, Sonpavde G, Lo Vullo S, et al. Nomogram-based prediction of overall survival in patients with metastatic urothelial carcinoma receiving first-line platinum-based chemotherapy: retrospective international study of invasive/advanced cancer of the urothelium (RISC). *Eur Urol* 2017;71:281-9. DOI PubMed PMC
63. Nelson AA, Cronk RJ, Lemke EA, et al. Early bone metastases are associated with worse outcomes in metastatic urothelial carcinoma. *BLC* 2021;7:33-42. DOI