REVIEW

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Advances in preclinical assessment of therapeutic targets for bladder cancer precision medicine

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Purpose of review
Bladder cancer incidence is on the rise, and until recently, there has been little to no change in treatment regimens over the last 40 years. Hence, it is imperative to work on strategies and approaches to untangle the complexity of intra- and inter-tumour heterogeneity of bladder cancer with the aim of improving patient-specific care and treatment outcomes. The focus of this review is therefore to highlight novel targets, advances, and therapy approaches for bladder cancer patients.

Recent findings
The success of combining an antibody-drug conjugate (ADC) with immunotherapy has been recently hailed as a game changer in treating bladder cancer patients. Hence, interest in other ADCs as a treatment option is also rife. Furthermore, strategies to overcome chemoresistance to standard therapy have been described recently. In addition, other studies showed that targeting genomic alterations (e.g. mutations in *FGFR3*, DNA damage repair genes and loss of the Y chromosome) could also be helpful as prognostic and treatment stratification biomarkers. The use of single-cell RNA sequencing approaches has allowed better characterisation of the tumour microenvironment and subsequent identification of novel targets. Functional precision medicine could be another avenue to improve and guide personalized treatment options.

Summary
Several novel preclinical targets and treatment options have been described recently. The validation of these advances will lead to the development and implementation of robust personalized treatment regimens for bladder cancer patients.

Keywords
antibody-drug conjugate, bladder cancer, chemoresistance, precision medicine, tumour microenvironment

INTRODUCTION

The standard of care for locally progressed or metastatic muscle invasive bladder cancer (MIBC) patients has predominantly been cisplatin-based neoadjuvant chemotherapy (NAC) followed by radical cystectomy [1]. In recent years, immune checkpoint inhibitors (ICIs) directed at programmed cell-death protein 1 (PD-1) or its ligand (PD-L1) have become available in the clinics as second line or maintenance therapy [2,3]. Recently, enfortumab vedotin, a Nectin-4-targeted antibody-drug conjugate (ADC) coupled to the microtubule disrupting compound auristatin E, has been approved by the FDA (2019) and EMA (2023) up to the third-line treatment after platinum and ICI therapy [1]. More recently, a current clinical trial has shown therapeutic superiority over cisplatin-based neoadjuvant chemotherapy for the first-line administration of the combination of an ICI (pembrolizumab) with enfortumab vedotin, which has been granted

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KEY POINTS

- The clinical use of ADCs will transform standard therapy in bladder cancer patients.
- Investigation of resistance mechanisms will overcome treatment failures.
- Identification and validation of prognostic and predictive molecular biomarkers are an unmet clinical need.
- Compounds beyond ICI, which target the TME, show promising results in preclinical studies.
- Functional precision medicine is an avenue to improve and guide personalized treatment options.

breakthrough designation by the FDA for the treatment of metastatic bladder cancer and may significantly change therapy sequence of bladder cancer in the future [4▪▪].

However, in spite of all innovations, treatment of patients across diverse stages of bladder cancer is often still palliative and not all patients experience (long-term) benefit from therapy. Furthermore, apart from the fact that some molecular patient stratification correlates with therapeutic value, including *FGFR3* mutation status [2,3], there is still insufficient evidence for robust prognostic or predictive molecular markers in bladder cancer [1]. Histopathological substaging of bladder cancer tissue, after transurethral resection of bladder tumour (TURBT), still mainly dictates treatment regimens [1]. The aim of this review is therefore to evaluate and present recent key advances in preclinical and clinical studies of therapeutic targets and treatment options for bladder cancer (Fig. 1).

OVERCOMING CHEMORESISTANCE AND IMPROVEMENT OF STANDARD THERAPY

Despite the recent success of ICI and ADC strategies that will likely shift therapy lines in the near future, chemotherapy is expected to remain a pillar in bladder cancer management. However, many patients fail to respond to standard chemotherapy (Gemcitabine and cisplatin), due to the development of chemoresistance. Several drug resistance mechanisms have been described before [5], including a genome-wide CRISPR screen demonstrating the involvement of the heterogeneous nuclear ribonucleoprotein U (HNRNPU) in cisplatin resistance, in a panel of established bladder cancer cell lines. Mechanistically, HNRNPU expression correlates with cisplatin resistance by regulating the chromatin structure and the transcription of DNA-damage repair genes (DDR). Inhibition of HNRNPU could be a promising target to restore cisplatin sensitivity in advanced MIBC patients [6▪]. A similar finding was reported about the transcription factor ZBTB11. Targeting ZBTB11 increased the sensitivity of bladder cell lines to cisplatin by inhibiting the transcription of the RNA helicase DDX1, leading to the accumulation of R-loops and subsequent cell death [7].

Another study focused more on the plasticity of cancer cells during the course of resistance acquisition [8▪▪]. Chemoresistance to gemcitabine and cisplatin treatment leads to a partial squamous differentiation in murine and human MIBC. A multiomics approach revealed that the lysosomal cysteine proteinase cathepsin H (CTSH) is responsible for this phenotype. Pharmacological blockage of CTSH with the proteinase inhibitor E64 induces full squamous differentiation and cell death via pyroptosis. Mechanistically, this proposed therapy depends on the activation of the tumour necrosis factor pathway [8▪▪]. This concept of inducing differentiation in cancer cells could be an encouraging new way of treating chemoresistant MIBC.

A different approach was used by Ertl *et al.* [9▪]. A high-throughput drug screen containing over 1700 compounds identified the antimetabolite drug clofarabine as a potent antineoplastic compound in a panel of commercially available bladder cancer cell lines. In addition, in patient-derived xenograft (PDX) models, clofarabine also showed high antitumour activity [9▪]. Clofarabine and gemcitabine are both antimetabolite drugs with similar, but not identical way of action. The former is a purine and the latter a pyrimidine-analogue. Although direct comparison of the two drugs revealed a slightly enhanced activity of gemcitabine *in vitro*, PDX models showed the superiority of clofarabine over gemcitabine *in vivo*, by displaying higher anticancer efficacy and less toxic side effects (M. Gutmann, I. Ertl, *et al.*, unpublished data). Therefore, this finding has the potential to transform standard therapy for MIBC patients.

TARGETING GENOMIC ALTERATIONS

Bladder cancer finds itself among the most mutated human cancer types and major genomic alterations have been identified [2]. Further, MIBC has also been categorized into six distinctive molecular subtypes based on molecular profiling analysis and identified signatures [10]. Despite these excessive research efforts, the molecular subtypes of bladder cancer and known genomic alterations are still barely used to guide treatment regimens [3].

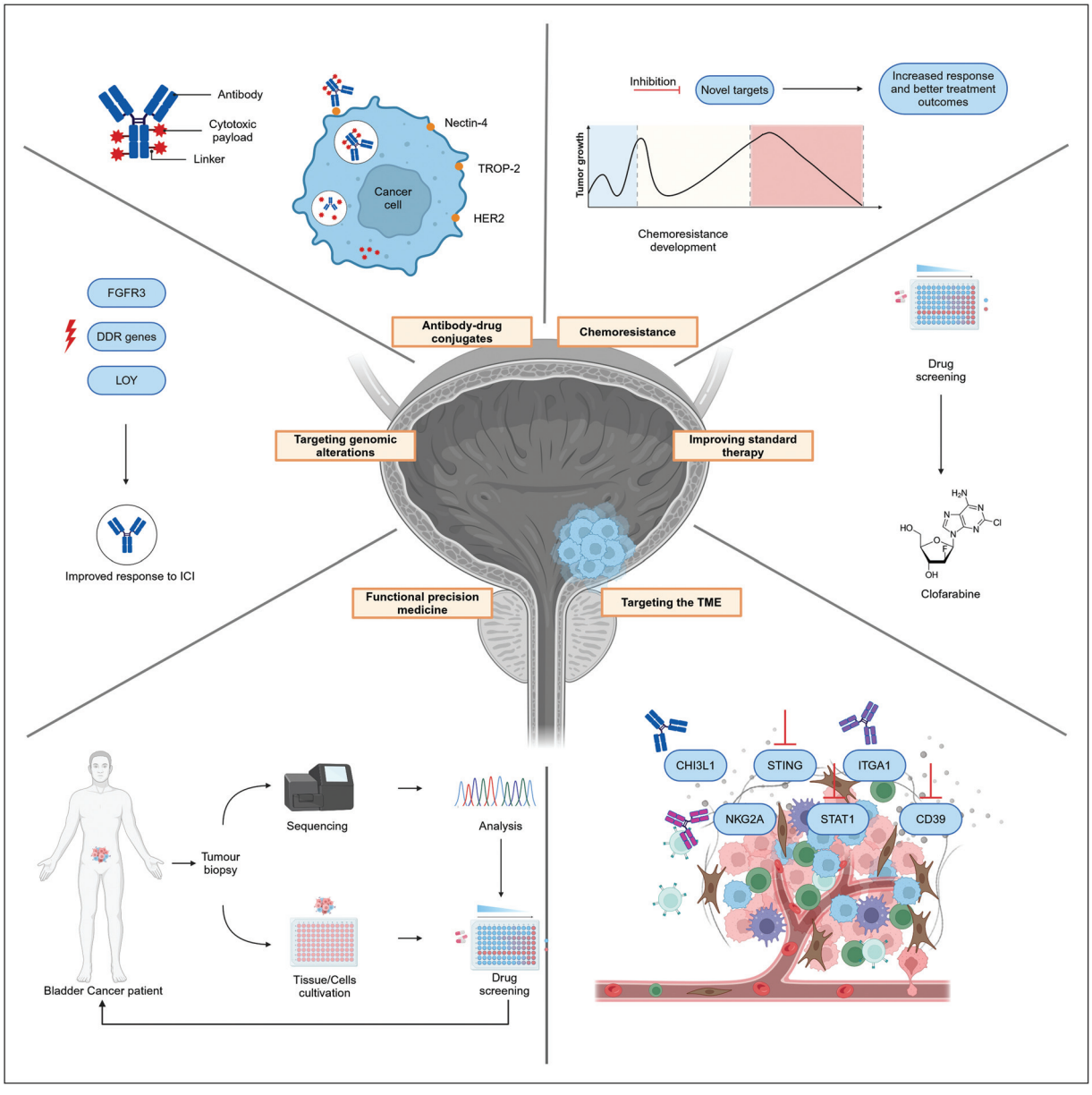

FIGURE 1. Graphical overview of recent advances in bladder cancer precision medicine.

It is well known that genes participating in DDR pathways and somatic alterations present in these genes correlate with better pathologic response to platinum compounds interfering with DNA replication and integrity. Deletion in the *ERCC2*, *RB1*, *ATM* and *FANCC* genes has been reported to correlate with patient response to cisplatin-based therapy, including NAC [11]. Other studies focused predominately on the role of the Ataxia-telangiectasia mutated (*ATM*) gene since it has an essential role in persevering genomic integrity and in the cellular response to DNA damage [12]. ATM loss increases the sensitivity of preclinical bladder cancer models to DNA repair-targeting agents, especially to ATR and PARP inhibitors. Moreover, the loss of ATM expression reshapes the tumour microenvironment (TME), conferring the sensitivity of the preclinical models to ICI therapy, while surprisingly having no effect in clinical cohorts [13▪]. In addition, Gil-Jimenez *et al.* [14▪] showed that only patients harbouring mutations in *ERCC2* had a better response to NAC, even though a positive association with recurrence-free or overall survival (OS) was not found. Thus, genetic alterations in DDR pathways can still not be used as a prognostic marker of the response rate of bladder cancer patients to chemotherapy.

Fibroblast growth factor receptor 3 (*FGFR3*) is often hyperactivated by point mutations and

amplified in MIBC [2]. The phase III clinical trial THOR revealed that erdafitinib, a pan-FGFR inhibitor, significantly improved progression-free survival (PFS), and OS in metastatic urothelial carcinoma patients with *FGFR* perturbations previously treated with ICI therapy [15]. Erdafitinib has been FDA-approved since 2019 for treating metastatic urothelial carcinoma and since January 2024 for treating locally advanced and/or metastatic urothelial carcinoma with *FGFR3* alterations even without previous ICI therapy [16]. Furthermore, in their recent study, Jin *et al.* [17▪▪] revealed a potential mechanism underlying the synergistic effect of ICI and erdafitinib that suggests a rationale for an immuno-targeted combination treatment for *FGFR3*-mutated bladder cancer: erdafitinib downregulates the expression of E3-ubiquitin ligase NEDD4, preventing it from targeting PD-L1 for ubiquitination. Furthermore, another study in a murine model of bladder cancer investigated cooperativity between ICI and FGFR inhibition. The results indicate a synergism where the FGFR inhibition with erdafitinib possibly reverses anti-PD-1 induced immunosuppression by abolishing Treg expansion [18].

Another major genomic alteration found in bladder cancer is the loss of the Y chromosome (LOY) [19]. LOY is connected to ageing and has been reported in various health conditions, including bladder cancer, where the loss rate varies between 10 and 40% and correlates with poor prognosis [20]. It was shown that Y^- tumours are more aggressive than their Y^+ counterparts *in vivo* in immune-competent hosts due to their ability to evade adaptive immunity by inducing $CD8^+$ T cell exhaustion in the TME. This phenotype is at least partly driven by the loss of the Y chromosome-encoded genes *UTY* and *KDM5D*. Patients with LOY have a better response to anti-PD1-targeted ICI, since it drives T cell differentiation from exhaustion to effector function [21▪▪]. This study proposed the use of LOY as a potential biomarker for the use of ICI in bladder cancer.

TARGETING THE TUMOUR MICROENVIRONMENT

The TME consists of a heterogeneous population of cancer, stromal and immune cells. The composition and the interplay of the TME can influence disease progression and therapy response, especially in the context of ICI therapy [22]. Furthermore, the broader availability of single-cell RNA sequencing (scRNA-seq) technologies has contributed to a better characterization of the TME in bladder cancer [23].

The importance of the TME composition was illustrated in a study describing overexpression of CD39 in bladder cancer. This ectonucleoside triphosphate diphosphohydrolase-1 converts extracellular ATP and ADP to immunosuppressive extracellular adenosine. Pharmacological inhibition of CD39 was able to reduce the growth of tumour cells in a murine orthotopic bladder cancer model. Furthermore, CD39 inhibition significantly increased the abundance of immune cells in the TME. Depletion of specific immune subpopulations showed that this effect depends on natural killer (NK) cells and conventional type 1 dendritic cells. Moreover, CD39 inhibition had a synergistic effect with cisplatin *in vivo*, suggesting the potential use of CD39 as a target in bladder cancer [24▪].

In addition, there is also increased research interest beyond the classical ICI targets (PD-1, PD-1L and CTLA-4) [25]. NKG2A (together with its co-receptor CD94) acts as an inhibitory receptor on NK and $CD8^+$ T cells when it binds to its ligand HLA-E (a nonclassical MHC class I molecule) [26]. Surprisingly, high NKG2A expression levels correlated with better survival and response to PD-L1 blockage in MIBC patients. Mechanistically, NKG2A+ PD1+ $CD8^+$ T cells use T cell receptor-independent innate-like function to exert antitumour activity. Nevertheless, in the context of high HLA-E expression in bladder cancer, pharmacological blockage of NKG2A enhances the cytotoxicity of T cells. This discovery could potentially also transform ICI therapy, by targeting the NKG2A/HLA-E interaction as an alternative immune checkpoint axis [27▪].

Cancer-associated fibroblasts (CAFs) are another important cell population in the TME of solid tumours. These highly heterogeneous stromal cells can play a tumour promoting or suppressing role in cancer [28]. A scRNA-seq analysis of bladder cancer from treatment-naive patients identified a novel subtype of CAFs in bladder cancer [29▪]. This subpopulation was characterized by expression of the urea transporter SLC14A1 and upregulation of interferon responsive genes. Therefore, this subpopulation was named interferon-regulated CAF (irCAF). The abundance of irCAFs correlated with poor prognosis and chemoresistance of bladder cancer patients. Furthermore, they also contributed to enhanced cancer stemness by releasing WNT5A to influence β-Catenin signalling in tumour cells in a paracrine way. On the other hand, chemotherapy can activate the cGAS-STING pathway that leads to the production of interferon and subsequently influences irCAF differentiation through STAT1. Thus, this finding supports the rationale to use STING or STAT1 inhibitors to improve response rates to chemotherapy in bladder cancer [29▪].

Another previously unknown CAF subset has been identified recently [30▪▪]. $PDGFRa^+$ $ITGA11^+$ CAFs play a major role in early-stage bladder cancer,

by aiding lymphovascular invasion and subsequent lymph node metastasis. Spatial transcriptomics revealed that PDGFRa$^+$ ITGA11$^+$ CAFs enhance extravasation of bladder cancer cells by binding to E-selectin (SELE) expressed on lymphatic endothelial cells. The release of the cytokine CHI3L1, which causes extracellular matrix remodelling, further enhances this phenotype. mAbs targeting ITGA11 and/or CHI3L1 significantly reduced lymphovascular invasion in a carcinogen-induced early-stage bladder cancer mouse model and a PDX model. Therefore, targeting PDGFRa$^+$ ITGA11$^+$ CAFs in early-stage bladder cancer patient could be an effective treatment option to reduce lymph node metastasis [30▪▪].

ANTIBODY-DRUG CONJUGATES

Many cytotoxic drugs cannot be used as chemotherapeutic agents in the clinics, because of their systemic side effects. The discovery of tumour-specific antigens led to the development of ADCs. This novel treatment approach uses mAbs coupled with a cytotoxic payload to specifically target and kill cancer cells [31]. As already mentioned above, approval of enfortumab vedotin with the anti-PD1 ICI pembrolizumab has the potential to change the standard of care for bladder cancer patients [4▪▪]. The success of this novel combination therapy might also partially be explained by the notion that Nectin-4 is a ligand of the inhibitory TIGIT receptor (T cell immunoreceptor with Ig and ITIM domains), expressed on most immune cells. Preclinical studies have shown that inhibitory Nectin-4 antibodies enhance tumour cell killing by blocking Nectin-4 TIGIT interactions NK cells [32]. The role of this novel immune checkpoint axis in bladder cancer has not been investigated yet.

Another ADC that was approved for bladder cancer patients is sacituzumab govitecan [33]. This ADC consists of an antibody targeting the transmembrane calcium signal transducer protein TROP-2, coupled with several molecules of SN-38, a topoisomerase-1 inhibitor. Several clinical trials are currently investigating the efficacy of using sacituzumab govitecan as a single agent or in combination with ICI [34]. Furthermore, a phase III trial to test the efficacy of sacituzumab govitecan as a single agent is ongoing [35]. Datopotamab deruxtecan (Dato-DXd) is an additional ADC, consisting of an antibody targeting TROP-2 and the topoisomerase-1 inhibitor deruxtecan, which showed potent antitumour activity in preclinical models of solid tumours [36]. Preliminary results from a phase 1 study demonstrated the tolerability, safety and encouraging efficacy of Dato-DXd in advanced or metastatic urothelial cancer patients [37]. Human epidermal growth factor 2 (HER2) is also overexpressed in a significant amount of MIBC patients [38]. Different ADCs targeting HER2 (disitamab vedotin, trastuzumab deruxtecan and trastuzumab emtasine) coupled with distinct cytotoxic compounds are also currently being investigated in clinical trials for their use in bladder cancer [34].

Despite the success rate of ADCs in the clinics, there is still some ambiguity about the use of ADC targets as prognostic biomarkers. One clinical trial detected uniformly high expression levels of Nectin-4 in a cohort of metastatic bladder cancer patients [39]. On the other hand, the recent analysis of Nectin-4 and TROP-2 protein expression in an advanced urothelial bladder cancer cohort showed that both ADC targets are widely detected via IHC, but their expression decreases in more aggressive subtypes (e.g. neuroendocrine-like MIBC) [40▪]. Furthermore, Nectin-4 protein expression was significantly lower in metastatic tumour samples compared to their patient-matched primary tumours. Downregulation of Nectin-4 in the metastatic tissue also correlated with a shortened PFS of metastatic patients in a multicentre enfortumab vedotin treated cohort [41▪]. It would therefore be beneficial to stratify patients according to their expression levels of target proteins before initiating ADC therapy. However, the identification and validation of robust additional biomarkers is required to predict responsiveness of ADCs beyond expression levels of target surface markers, especially in the context of metastatic disease where tissue material is usually scarce. Moreover, there will also be the clinical need to understand unresponsiveness to ADC treatments and to investigate potential resistance mechanisms.

FUNCTIONAL PRECISION MEDICINE

The lack of prognostic or predictive molecular markers is the prime cause of treatment failure in bladder cancer patients. Inter-patient heterogeneity further exacerbates this problem. Highly personalized drug screens, also known as functional precision medicine, could be an avenue to improve response rates to cancer treatments [42]. In hematologic cancers, functional precision medicine has already been shown to provide clinical benefit to patients [43]. Hence, efforts have been made to implement this approach in the field of bladder cancer, especially in the light of frequent poor fitness rendering patients ineligible for platinum-based chemotherapy. Patient-derived organoids that preserve molecular and phenotypic features of the primary tumour have been screened to determine their sensitivity to a panel of drugs. The

various response rates reinforced the notion of bladder cancer as a very heterogeneous disease [44▪]. A similar study used organoids from sarcomatoid urothelial bladder cancer patients to identify personalized vulnerabilities in this aggressive form of bladder cancer. Again, this organoid model resembled molecular and phenotypic characteristics of the primary tumour. A high-throughput screen with 1567 compounds revealed high sensitivity of these organoids against glucocorticoids, compared to organoids from other subtypes of bladder cancer [45]. Unfortunately, both studies reported potential vulnerabilities just retrospectively and were not designed to use these results in the treatment of the corresponding bladder cancer patients. Further, despite technological advances, screening of patient-derived organoids is still expensive and requires trained personnel and equipment. In addition, difficulties in cultivating cancer cells with their TME *ex vivo* is also an obstacle to implement this approach as standard of care in the clinics for solid tumours. Nevertheless, functional precision therapy could be an avenue to guide treatment options in advanced and metastatic bladder cancer in the future.

CONCLUSION

The recent presentation of promising preliminary data from a phase III clinical trial combining enfortumab vedotin with pembrolizumab [4▪▪] caused a buzz in the field of uro-oncology. This finding will undoubtedly transform the management of advanced and metastatic bladder cancer. Nevertheless, further research will be needed to assess limitations (e.g. development of resistance) and to identify patients that will benefit most from this novel treatment approach. Further, the search for prognostic or predictive biomarkers in bladder cancer patients will continue. Technological advances in scRNA-seq [23] and also spatial transcriptomics [46] will aid in the identification and validation of molecular markers. In addition, advancements in detecting circulating and urinary tumour DNA in liquid biopsies (plasma and urine) will further improve the care, diagnosis and monitoring of bladder cancer patients [47]. These recent advances, together with the future development of fast and cost-effective drug screening methods [42], will hopefully lead to the generation of robust guidelines for personalized treatment options.

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Conflicts of interest

There are no conflicts of interest.

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