



Review

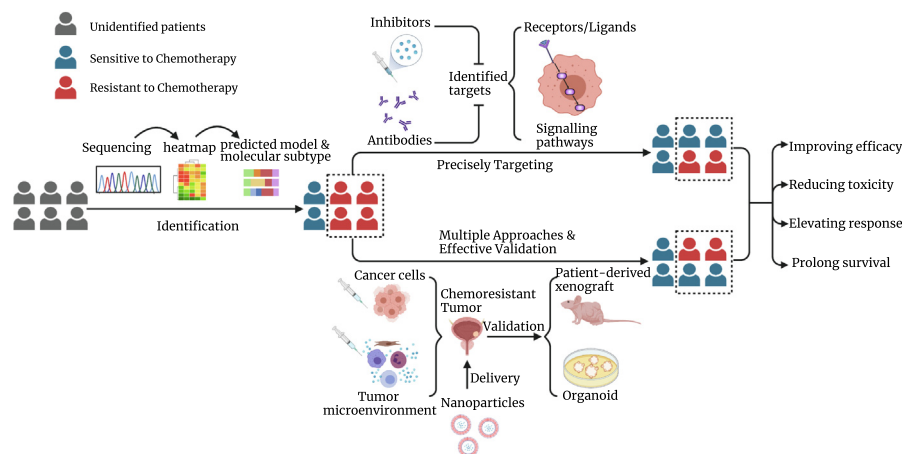
Emerging strategies for the improvement of chemotherapy in bladder cancer: Current knowledge and future perspectives

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HIGHLIGHTS

- The response of chemotherapy and prognosis in bladder cancer is unsatisfied.
- Immunotherapy, targeted therapy, and ADC improve the efficacy of chemotherapy.
- Emerging targets in cancer cells and TME spawned novel preclinical agents.
- Novel drug delivery, such as nanotechnology, enhances effects of chemotherapeutics.
- The organoid and PDX model are promising to screen and evaluate the target therapy.

GRAPHICAL ABSTRACT



Abbreviations: ADC, antibody-drug conjugate; AR, androgen receptor; ARA, arachidonic acid; AUC, area under the curve; BCG, bacilli Calmette-Guerin; CAF, cancer-associated fibroblast; CEBPD, CCAAT/enhancer-binding protein delta; CHK1, checkpoint kinase 1; CK1 δ , casein kinase 1 delta; COX-2, cyclooxygenase-2; CR, complete response; CSCs, cancer stem cells; CYP450, cytochrome P450 epoxygenases; dCK, deoxycytidine kinase; EET, epoxyeicosatrienoic acids; EGFR, epidermal growth factor receptor; ER β , estrogen receptor beta; EV, enfortumab vedotin; FDA, Food and Drug Administration; FGF9, fibroblast growth factor 9; FGFR3c, fibroblast growth factor 3c; GC, gemcitabine and cisplatin; H3K27me3, trimethylation of lysine 27 on histone H3 protein subunit; H3K4me3, trimethylation of lysine 4 on histone H3 protein subunit; Her2, Human epidermal growth factor receptor 2; hnRNP, heterogeneous nuclear ribonucleoprotein K; HSP90, heat shock protein 90; ICIs, immune checkpoint inhibitors; IGF-1, insulin-like growth factor-1; IGF-1R, insulin-like growth factor-1 receptor; Maspin, Mammary serine protease inhibitor; MIBC, muscle invasive bladder cancer; MMAE, monomethyl auristatin E; MVAC, methotrexate, vinblastine, doxorubicin and cisplatin; NMIBC, non-muscle invasive bladder cancer; ORR, objective response rate; OS, overall survival; PARP, Poly (adenosine diphosphate [ADP]) ribose polymerases; PDGF-BB, platelet-derived growth factor-B dimer; PDGFR, platelet-derived growth factor receptor; PDX, patient-derived xenograft; PFS, progression-free survival; PGE₂, prostaglandin E2; ROC, receiver operator characteristic; sEH, soluble epoxide hydrolase; SG, sacituzumab govitecan; TACC3, transforming acidic coiled-coil protein 3; TEAD, TEA domain transcription factor; TME, tumor microenvironment; VEGF, vascular endothelial growth factor; VEGFR-2, vascular endothelial growth factor receptor-2; WDR5, WD repeat domain 5; YAP, yes associated protein.

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ABSTRACT

Background: Chemotherapy is a first-line treatment for advanced and metastatic bladder cancer, but the unsatisfactory objective response rate to this treatment yields poor 5-year patient survival. Only PD-1/PD-L1-based immune checkpoint inhibitors, FGFR3 inhibitors and antibody-drug conjugates are approved by the FDA to be used in bladder cancer, mainly for platinum-refractory or platinum-ineligible locally advanced or metastatic urothelial carcinoma. Emerging studies indicate that the combination of targeted therapy and chemotherapy shows better efficacy than targeted therapy or chemotherapy alone. Newly identified targets in cancer cells and various functions of the tumour microenvironment have spawned novel agents and regimens, which give impetus to sensitizing chemotherapy in the bladder cancer setting.

Aim of Review: This review aims to present the current evidence for potentiating the efficacy of chemotherapy in bladder cancer. We focus on combining chemotherapy with other treatments as follows: targeted therapy, including immunotherapy and antibody-drug conjugates in clinic; novel targeted drugs and nanoparticles in preclinical models and potential targets that may contribute to chemosensitivity in future clinical practice. The prospect of precision therapy is also discussed in bladder cancer.

Key Scientific Concepts of Review: Combining chemotherapy drugs with immune checkpoint inhibitors, antibody-drug conjugates and VEGF inhibitors potentially elevates the response rate and survival. Novel targets, including cancer stem cells, DNA damage repair, antiapoptosis, drug metabolism and the tumour microenvironment, contribute to chemosensitization. Gene alteration-based drug selection and patient-derived xenograft- and organoid-based drug validation are the future for precision therapy.

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Current status of chemotherapy in bladder cancer

Bladder cancer is the most common cancer of the urinary system with an estimated 573,000 new cases and 212,000 deaths occurring annually worldwide [1]. Emerging urine assays facilitate early detection and risk stratification of bladder cancer [2,3]. Three-quarters of newly diagnosed bladder cancers are non-muscle invasive bladder cancers (NMIBCs), which are usually treated by transurethral resection followed by intravesical chemotherapy or bacilli Calmette–Guerin (BCG) therapy [4]. Muscle invasive bladder cancer (MIBC) accounts for the remaining newly diagnosed patients and may also arise from 10 to 20% of NMIBC cases that eventually progress [5]. As an aggressive disease, early radical cystectomy plus pelvic node dissection remains the basic management for MIBC. However, the 5-year cancer-specific mortality rate of bladder cancer patients has not notably decreased over the past 3 decades, indicating the limitations of the current therapeutic approaches [6].

Platinum-based chemotherapy, as the first-line management strategy against advanced urothelial cancer, is supported by level 1 evidence [7–10]. The most common regimens for bladder cancer are the combination of gemcitabine and cisplatin (GC) as well as the combination of methotrexate, vinblastine, doxorubicin and cisplatin (MVAC). GC has been demonstrated to have comparable efficacy, better safety and tolerability compared to MVAC [9,10]. For patients who have undergone neoadjuvant chemotherapy (i.e., chemotherapy before operation), platinum-based regimens achieve a complete response (CR) rate of 23.9–24.5%, barely improving the 5-year overall survival (OS) from 45% to 50% compared to local treatment alone [8,11]. Nonresponders gain limited benefit from neoadjuvant chemotherapy due to suffering from the toxicity of chemotherapy drugs and delaying surgery, resulting in a decrease in 5-year cancer-specific survival from 90% to 30–40% compared to responders [8,12,13]. For metastatic bladder cancers, the traditional regimens of chemotherapy exhibit relatively high objective response rates of 36–65%. However, the response does not translate to better survival stats with only 13–15% for the 5-year OS, indicating that chemoresistance appears in almost all patients as the disease progresses [6,14,15]. The prognosis for

metastatic patients who do not respond or recur after first-line chemotherapy is extremely poor, yet no alternative approaches have been developed [16]. Based on current knowledge, resistance to chemotherapy agents largely limits the efficacy of the existing standards in bladder cancer. Limited tolerability also notably restricts the application of chemotherapy. Thus, novel combined therapeutic strategies are required to improve the efficacy and decrease the side effects of chemotherapy in bladder cancer.

Targeted therapies are widely used and show satisfactory therapeutic effects in many cancers, such as breast, lung and colon cancers [17–19]. However, only PD-1/PD-L1-based immune checkpoint inhibitors (ICIs), FGFR3 inhibitors and antibody-drug conjugates (ADCs) are approved by the FDA for the management of advanced/metastatic urothelial carcinoma in platinum-refractory or platinum-ineligible patients. Emerging studies indicate that the combination of targeted therapy and chemotherapy shows better efficacy than targeted therapy or chemotherapy alone [20–22]. This review highlights the therapeutic approaches that potentiate the effect of chemotherapy in bladder cancer. We focus on combining chemotherapy with the following other treatments: targeted therapy, including immunotherapy and antibody-drug conjugates in the clinic; novel targeted drugs and nanoparticles in preclinical models; and potential targets that may contribute to chemosensitivity in future clinical practice. We also briefly discuss the potential of identifying target patients by sequencing and gene expression models as well as by evaluating chemosensitivity via patient-derived xenografts (PDXs) and organoids, which may provide evidence for the precision management and improvement of the chemotherapy efficacy of bladder cancer.

Improvement of chemotherapy in clinical trials

Clinical trials are ongoing to provide high-level evidence of different regimens potentiating the efficacy of chemotherapy in bladder cancer. The reported regimens that improve chemosensitivity include combining cytotoxic drugs with other agents, such as ICIs, targeted agents and ADCs (Table 1).

Table 1
Completed and reported clinical trials of combination of targeted drugs and chemotherapy in urothelial carcinoma.

Drug	Category	Phase	Characteristics of the participants	Intervention	Pts	Outcomes [Median (95% CI)]			NCT No./Ref.
						PFS (months)	OS (months)	ORR (%)	
<i>Immune checkpoint inhibitors</i>									
Atezolizumab	anti-PD-L1 antibody	III	Locally advanced or metastatic urothelial carcinoma	A + C: Standard chemotherapy† + Atezolizumab A: Atezolizumab	451 362	8.2 (6.5 to 8.3) NA	16.0 (13.9 to 18.9) NA	47 (43 to 52) 23 (19 to 28)	NCT02807636, [26]
				C: Placebo + standard chemotherapy† p-value	400	6.3 (6.2 to 7.0) A + C vs C p = 0.007	13.4 (12.0 to 15.2) A + C vs C p = 0.027	44 (39 to 49) NA	
Pembrolizumab	anti-PD-1 antibody	III	Advanced or metastatic urothelial carcinoma	P + C: Standard chemotherapy† + Pembrolizumab P: Pembrolizumab C: Standard chemotherapy† p-value	351 307 302	8.3 (7.5 to 8.5) 3.9 (2.3 to 5.1) 7.1 (6.4 to 7.9)	17.0 (14.5 to 19.5) 15.6 (12.1 to 17.9) 14.3 (12.3 to 16.7)	54.7 (49.3 to 60.0) 30.3 (25.2 to 35.8) 44.9 (39.6 to 50.2) NA	NCT02853305, [27]
Ipilimumab	anti-CTLA-4 antibody	II	Chemotherapy-naïve patients with metastatic urothelial cancer	GC + Ipilimumab	36	7.9 (6.4 to 9.9)	13.9 (10.5 to 23.4)	69 (NA)	NCT01524991, [28]
<i>Targeted drugs</i>									
Ramucirumab	anti-VEGFR-2 antibody	III	Locally advanced / unresectable / metastatic urothelial carcinoma who progressed on or after platinum-based therapy	R + D: Ramucirumab + Docetaxel D: Placebo + Docetaxel p-value	263 267	4.07 (2.96 to 4.47) 2.76 (2.60 to 2.96)	9.40 (7.89 to 11.43) 7.85 (7.00 to 9.30)	24.5 (18.8 to 30.3) 14.0 (9.4 to 18.6) NA	NCT02426125, [32]
Bevacizumab	anti-VEGF antibody	II	Untreated or relapsed locally advanced or metastatic transitional cell carcinoma of the bladder	GC + Bevacizumab	45	8.2 (6.8 to 10.3)	19.1 (12.4 to 22.7)	72 (NA)	NCT00234494, [33]
Bevacizumab	anti-VEGF antibody	II	Locally Advanced Urothelial Cancer	Neoadjuvant ddmVAC + Bevacizumab	60	NA	5-year OS rate: 63% (51% to 77%)	53 (NA)	NCT00506155, [34]
Cetuximab	anti-EGFR antibody	II	Metastatic, locally recurrent, or unresectable urothelial carcinoma	Treatment: GC + Cetuximab Control: GC p-value	60 29	7.6 (6.1 to 8.7) 8.5 (5.7 to 10.4) NA	14.3 (11.6 to 22.2) 17.4 (12.8 to NA) NA	61.4 (48 to 74) 57.1 (37 to 76) NA	NCT00645593, [35]
<i>Antibody-drug conjugates</i>									
Enfortumab Vedotin	Conjugate of anti-Nectin-4 antibody and MMAE	I	Metastatic urothelial carcinoma progressed on chemotherapy, or ineligible for cisplatin	Enfortumab Vedotin monotherapy	155	5.4 (5.1 to 6.3)	12.3 (9.3 to 15.3)	43 (33.6 to 52.6)	NCT02091999, [39]
Sacituzumab Govitecan	Conjugate of anti-TROP-2 antibody and SN-38	II	Locally advanced or unresectable or metastatic Urothelial Carcinoma progressed chemotherapy and ICIs	Sacituzumab Govitecan monotherapy	113	5.4 (3.5 to 7.2)	10.9 (9.0 to 13.8)	27 (19.5 to 36.6)	NCT03547973, [41]
RC48-ADC	Conjugate of anti-Her2 antibody and MMAE	II	HER2-positive patients with locally advanced or metastatic urothelial carcinoma	RC48-ADC monotherapy	43	NA	NA	60.5 (44.4 to 75.0)	NCT03507166, [42]

†The regimen of standard therapy is gemcitabine + cisplatin/carboplatin.

*The designed p value boundary was 0.0019.

**The designed p value boundary was 0.0142.

Pts, Patients; PFS, progression-free survival; OS, overall survival; ORR, objective response rate; NA, not available; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; EGFR, epidermal growth factor receptor; GC, gemcitabine plus cisplatin; ddmvac, dose-dense Methotrexate, Vinblastine, Adriamycin and Cisplatin; MMAE, monomethyl auristatin E; ICIs, immune checkpoint inhibitors.

Immune checkpoint inhibitors (ICIs)

ICIs, including PD-1 and PD-L1 inhibitors, have been approved as second-line treatments after traditional chemotherapy in locally advanced or metastatic urothelial carcinoma for years [23–25]. It is also currently being assessed whether immune checkpoint inhibitors improve the efficacy of platinum-based chemotherapy (Table 1). In the IMvigor130 phase 3 trial, Galsky *et al* supported the use of the anti-PD-L1 agent, atezolizumab, combined with platinum-based chemotherapy as a first-line treatment option for metastatic urothelial carcinoma. [26] Specifically, combining atezolizumab with platinum prolonged the median progression-free survival (PFS) from 6.3 months to 8.2 months ($p = 0.007$) and improved the median OS from 13.4 months to 16.0 months ($p = 0.027$) compared to chemotherapy alone. Conversely, the KEYNOTE-361 phase 3 trial compared the efficacy of first-line pembrolizumab (a PD-1 inhibitor) plus chemotherapy versus chemotherapy for advanced urothelial carcinoma [27]. The final data showed that the addition of pembrolizumab to chemotherapy did not improve the median PFS (8.3 months vs. 7.1 months, $p = 0.0033 >$ designed p value boundary of 0.0019) or median OS (17.0 months vs. 14.3 months, $p = 0.0407 >$ designed p value boundary of 0.0142) [27]. Furthermore, a multicentre phase 2 study showed a response rate of 69% and 1-year OS of 61% in the treatment of 2 cycles of GC followed by 4 cycles of GC plus ipilimumab, a CTLA-4 inhibitor, in 36 patients with metastatic urothelial carcinoma [28].

Combination therapy with two ICIs is the latest investigation of immunotherapy against bladder cancer. Combining ipilimumab with nivolumab (another PD-1 inhibitor) has been reported in a multicohort study to have sustained antitumour activity in platinum-pretreated metastatic urothelial carcinoma patients (ORR 26.9% to 38.0% for different doses) [29]. Another phase 3 study has assessed the efficacy of durvalumab (a PD-L1 inhibitor) combined with tremelimumab (a CTLA-4 inhibitor) as the first-line approach to treat metastatic urothelial carcinoma. The study reported a median OS of 15.1 months in the durvalumab plus tremelimumab group versus 12.1 months in the chemotherapy group ($p = 0.075$), which did not meet its coprimary endpoints [30]. Interestingly, nivolumab plus ipilimumab combined with chemotherapy provided an improvement in OS versus monochemotherapy (14.1 months vs. 10.7 months, $p = 0.00065$) in a phase 3 trial of non-small-cell lung cancer. These results may encourage researchers to validate whether this regimen could be applied as a novel treatment option for urothelial carcinoma [31]. These aforementioned trials provide feasible approaches for combining chemotherapy plus immunotherapy to treat metastatic urothelial cancer. However, additional clinical trials are required to determine whether ICIs plus chemotherapy can be regarded as a first-line treatment for advanced urothelial carcinoma.

Targeting VEGF, the EGFR pathway and DNA methyltransferase

Since targeted therapy was introduced, the combination of chemotherapeutic agents and targeted drugs has been considered to improve the curative effect of bladder cancer (Table 1). Adding ramucirumab, an inhibitor of VEGFR-2, to docetaxel has been evaluated to treat patients with locally advanced or metastatic disease who progressed on or after platinum-based chemotherapy; the median PFS was significantly improved with ramucirumab plus docetaxel compared to placebo plus docetaxel (4.07 vs. 2.76 months, $p = 0.0002$), but the median OS was not significantly improved (9.4 vs. 7.9 months, $p = 0.25$) [32]. Thus, the actual benefit might be restricted for the addition of ramucirumab to docetaxel in an unselected population of platinum-refractory advanced urothelial carcinoma.

Bevacizumab, a recombinant monoclonal antibody against vascular endothelial growth factor (VEGF), which was added to GC, has been identified to achieve a response rate of 72% and a complete response rate of 19% in a phase 2 trial [33]. Moreover, bevacizumab has also been explored in combination with MVAC for neoadjuvant treatment for locally advanced bladder cancer [34]. In this phase 2 trial, the rate of downstaging to \leq pT1N0 was 53%, and the 5-year OS was 63% for bevacizumab, exhibiting no appreciable impact on outcomes. Interestingly, the authors identified improved survival in patients with the basal subtype compared to luminal and p53-like tumours (5-year OS 91%, 73% and 36%, $p = 0.015$), suggesting that gene expression profiles differ in the initial sensitivity to chemotherapy in bladder cancer.

Another promising targeted drug, cetuximab, an inhibitor of epidermal growth factor receptor (EGFR), has been combined with GC for the treatment of advanced urothelial carcinoma in a phase 2 trial [35]. However, cetuximab plus GC exhibited no improvements in outcomes but was associated with more adverse effects.

In addition, a DNA methyltransferase inhibitor has been introduced to reverse cisplatin resistance in urothelial carcinoma cell line models [36,37]. A recent phase 1 trial established a tolerable dose of guadecitabine, a DNA methyltransferase inhibitor, in combination with GC for further evaluation of the efficacy [38]. Taken together, targeted therapy has shown a remarkable improvement on chemotherapy in bladder cancer, especially bevacizumab. Additional clinical studies are required to validate and support this hypothesis.

Antibody-drug conjugate (ADC) is a novel target chemotherapy

ADC has been constructed to precisely deliver cytotoxic agents or inhibitors towards cancer cells, depending on the specific antibody that binds to the surface antigen of cancer cells. Importantly, ADC is considered a cutting edge therapy for bladder cancer management (Table 1). Recently, in December 2019, the US Food and Drug Administration (FDA) approved enfortumab vedotin (EV) for bladder cancer treatment, becoming the landmark of ADC application in bladder cancer [21]. EV is a conjugate of monoclonal antibody against Nectin-4, which is overexpressed in urothelial carcinomas, and a microtubule-disrupting agent, monomethyl auristatin E (MMAE). In a phase 1 trial that enrolled 155 metastatic patients who failed chemotherapy, EV treatment exhibited an objective response rate (ORR) of 43%, a median OS of 12.3 months and a 1-year OS rate of 51.8%, while grade \geq 3 adverse effects occurred in 34% of participants [39]. EV is now being evaluated in combination with ICIs and/or chemotherapy agents in patients with locally advanced/metastatic urothelial carcinoma (NCT03288545). In addition, another ADC has been constructed that conjugates MMAE with an anti-SLITRK6 antibody, and this ADC is termed ASG-15ME [40]. A phase 1 trial (NCT01963052) of ASG-15ME given as monotherapy for metastatic urothelial cancer was completed in 2020, and the results have not yet been reported.

The second approved ADC for bladder cancer treatment is sacituzumab govitecan (SG), which conjugates an anti-Trop-2 monoclonal antibody with SN-38, the active metabolite of irinotecan. In the latest phase 2 clinical trial, 113 advanced/metastatic urothelial carcinoma patients who had received ineffective ICI treatment or chemotherapy were enrolled. SG treatment in this trial exhibited an ORR of 27%, a median PFS of 5.4 months and a median OS of 10.9 months with 6% of participants terminating treatment because of treatment-related adverse events [41].

Human epidermal growth factor receptor 2 (Her2) is a widely known tyrosine kinase receptor that is expressed in many solid tumours, including bladder cancer. Targeting Her2 has been studied for decades, while anti-Her2 ADCs are also being explored and assessed in clinical trials. RC48-ADC is a novel humanized

anti-Her2 antibody connected with MMAE. In a phase 2 clinical trial started in December 2017, 43 Her-2-positive patients with advanced/metastatic urothelial carcinoma received RC48-ADC monotherapy [42]. By the end of January 2019, the ORR was 60.5%, and the ORR was 64.9% in patients with visceral metastasis and 70.0% in those with liver metastasis; the OS and PFS was not reached [42]. These favourable results encouraged us to keep notice of the final outcomes of the completed phase 2 trial (NCT03507166). Trastuzumab emtansine (TDM1) consists of emtansine, an anti-microtubule, and the anti-Her2 antibody, trastuzumab, which has been approved by the FDA for breast cancer patients. TDM1 has been reported to have antitumour effects in preclinical models of HER2-overexpressing bladder cancer [43]. However, the clinical trial of TDM1 to treat urothelial carcinoma did not report a favourable result (NCT02999672). Trastuzumab deruxtecan (DS-8201a) comprises a trastuzumab conjugated with an exatecan derivative, a topoisomerase I inhibitor, which has a higher drug-to-antibody ratio to potentially target Her2 low-expressed tumours compared to TDM1. A phase 1 clinical trial for the combination of trastuzumab deruxtecan and nivolumab in advanced bladder cancer is ongoing (NCT03523572).

Promising strategies of chemosensitization in preclinical studies

As mentioned above, targeted therapy is important in chemosensitization in bladder cancer. Targeted therapy and immunotherapy are mainly investigated to target cancer cells and/or immune cells that are not under the pressure of chemotherapeutic agents. Intriguingly, chemotherapy stress drives different heterogeneity in cancer cells and/or immune cells from those unexposed to chemotherapy [44,45]. It is reasonable to assess whether the introduced targeted therapy and/or immunotherapy could expand their use to improve the efficacy of chemotherapy. Novel molecules and pathways that were identified to specifically contribute to chemosensitivity warrant more attention to determine their translational value. Novel phenotypes discovered in recent years, such as cancer stem cells, inflammation pathways and tumour immunology, have shown the potential of sensitizing bladder cancer cells to chemoresistance in animal models. Newly identified regulatory mechanisms of traditional phenotypes of chemoresistance, including drug metabolism, DNA damage repair pathways, oncogenes and tumour suppressors, which have been validated *in vivo*, provide novel ideas. Furthermore, nanotechnology modifies the pharmacokinetics of chemotherapy drugs in animal models. These findings encourage clinical trials to investigate the potential of agents targeting these molecules and pathways and to apply nanotechnology to improve the outcome of chemotherapy against advanced bladder cancer (Table 2, Fig. 1 and Fig. 2).

Targeting cancer stem cells

Cancer stem cells (CSCs) are tumour-initiating cells that are characterized by self-renewal and differentiation, conserving the heterogeneity of cancer cells [46]. CSCs have been reported to have an advantage in surviving under the pressure of chemotherapy, which is one of the main reasons for chemoresistance [47]. Targeting CSCs may be a promising protocol to improve the response to chemotherapy and has recently been evaluated in preclinical studies (Fig. 1). Tatokoro *et al* isolated CSCs based on CD44 (a marker of CSCs)-positive cells and found that they are more resistant to cisplatin than the non-CSC subgroups [48]. These researchers also found that 17-DMAG, an HSP90 inhibitor, potentiates the cytotoxicity of cisplatin against CSCs by enhancing cisplatin-induced

apoptosis, which was further confirmed in xenografts *in vivo* [48]. In addition, yes-associated protein (YAP), the effector of the Hippo pathway, plays a key role in CSC self-renewal and expansion in several cancers. YAP drives the self-renewal of CSCs that express OV6, which is also a CSC surface marker [49]. Mechanistically, an autocrine regulatory loop shows that YAP activates PDGFB transcription, while PDGF-BB is secreted and binds to its receptor, PDGFR, to stabilize YAP. Vertepofin (a YAP inhibitor) and CP-673451 (a PDGFR inhibitor) have been applied to interrupt the autocrine regulatory loop, resulting in alleviation of the chemoresistance of OV6-positive CSCs to cisplatin *in vivo* [49].

Targeting inflammation pathway

Kurtova *et al* revealed a novel mechanism, in which chemotherapy-induced inflammation impedes chemoresistance [50]. Specifically, chemotherapy effectively induces apoptosis and then releases prostaglandin E₂ (PGE₂), an inflammatory cytokine. Chemotherapy-associated PGE₂ releases repopulated CSCs from a quiescent label-retaining pool into cell division, which is similar to the mobilization of normal stem cells during wound repair (Figs. 1 and 2). The administration of the cyclooxygenase-2 (COX2) inhibitor, celecoxib, abrogates the PGE₂-mediated wound response and weakens the aggressive character of chemoresistance in xenograft models [50]. However, targeting PGE₂ with COX-2-selective inhibitors has achieved unsatisfactory results in human clinical trials to enhance the efficacy of chemotherapy in several cancers [51–53]. Furthermore, epoxyeicosatrienoic acids (EETs) are also derived from the arachidonic acid pathway but have anti-inflammatory functions [54,55] and are decomposed to inactive forms by soluble epoxide hydrolase (sEH) [56]. EET inhibits the transcription of COX-2, and thus, less PGE₂ is produced. Therefore, sEH inhibitors enhance the anti-inflammatory effects of COX-2-selective inhibitors [57]. The strategy of dual inhibition of COX-2/sEH has also shown the potential of enhancing the antitumour efficacy of chemotherapeutic agents. Specifically, a recent study has reported that PTUPB, a compound that inhibits both COX-2 and sEH, potentiates cisplatin efficacy in bladder cancer cell lines and improves the response to GC in patient-derived xenografts by promoting apoptosis and abrogating activation of the MAPK/ERK and PI3K/Akt pathways (Fig. 2) [58].

Targeting drug transportation and metabolism

The transporter and metabolizing enzymes of chemotherapeutic agents reduce the effective drug concentration and, thus, implicate potential effects of chemosensitivity (Fig. 2). Kita *et al* conducted a high-throughput screening of drugs to identify chemosensitivity-related compounds [59], and they identified disulfiram, an anti-alcoholism drug, to enhance the efficacy of cisplatin by affecting the localization of ATP7A, a cisplatin efflux transporter. In patient-derived and cell-based xenograft models, disulfiram nanoparticles have been demonstrated to show synergistic effects with cisplatin and present a well-characterized safety profile, providing the potential to repurpose disulfiram in clinical practice [59]. For gemcitabine, regulation of its rate-limiting metabolizing enzyme, deoxycytidine kinase (dCK), which phosphorylates gemcitabine to functional forms, also plays an important role in sensitizing the anticancer efficacy. SR-3029 inhibits casein kinase 1 delta (CK1 δ), resulting in the upregulation of dCK and synergistic activity to enhance apoptosis in pancreatic and bladder cancer [60]. However, the efficacy of the combination treatment of CK1 δ inhibitor and gemcitabine has been verified in the pancreatic tumour model *in vivo* but not in the bladder cancer model.

Table 2
Preclinical drugs and inhibitors that sensitize chemoresistance in bladder cancer.

Regulation category	Pathways/phenotypes	Targets	Drugs/Inhibitors	In vivo experiment	Ref.
CSCs	CSCs apoptosis	HSP90	17-DMAG (<i>in vitro</i>) 17-AAG (<i>in vivo</i>)	5637 Xenograft	[48]
CSCs	YAP/TEAD1/PDGF-BB/PDGFR loop-OV6	YAP, PDGFR	Vertepofin (YAP inhibitor) CP-673451 (PDGFR inhibitor)	Orthotopic model of OV6 + cells	[49]
CSCs and inflammation	PGE ₂ /COX2-mediated CSCs repopulation	COX2	Celecoxib	T24 Xenograft and PDX	[50]
	EET/COX-2/PGE ₂	COX-2 and sEH	PTUPB	PDX	[58]
Cisplatin transportation	Localization of ATP7A	–	disulfiram	PDX and PDX-derived organoid	[59]
Gemcitabine metabolism	CK1δ-dCK	CK1δ	SR-3029	Pancreatic tumor model	[60]
DNA damage repair, anti-apoptosis, Tumor immunology	WDR5-MLL complex mediated H3K4me3, PD-L1 based immune invasion	WDR5	OICR-9429	UM-UC-3 xenograft	[67]
DNA damage repair	Nucleotide excision repair	ERCC2 mutation	–	Orthotopic model of ERCC2 WT and Mut cells	[66]
Oncogenes	autophagy and cell senescence in cells with HRAS mutation	–	pterostilbene	T24 Xenograft	[72]
Tumor suppressors Receptors	P73 accumulation-GC-induced apoptosis	–	1,25D ₃	T24 Xenograft	[74]
	EGFR/STAT3/CEBPD/ABCB1&ABCC2	EGFR, STAT3	Gefitinib (EGFR inhibitor) S3I-201 (STAT3 inhibitor)	NTUB1/P xenograft	[76]
Receptors	AR	AR	ASC-J9	J82 Xenograft	[77]

CSCs, cancer stem cells; PDX, patient-derived xenograft; HSP90, heat shock protein 90; YAP, yes associated protein; TEAD, TEA domain transcription factor; PDGF-BB, platelet-derived growth factor-B dimer; PDGFR, platelet-derived growth factor receptor; PGE₂, prostaglandin E₂; EET, epoxyeicosatrienoic acids; sEH, soluble epoxide hydrolase; COX-2, cyclooxygenase-2; CYP2, cytochrome P450 epoxygenases; CK1δ, casein kinase 1 delta; dCK, deoxycytidine kinase; WDR5, WD repeat domain 5; H3K4me3, trimethylation of lysine 4 on histone H3 protein subunit; EGFR, epithelial growth factor receptor; CEBPD, CCAAT/enhancer-binding protein delta; AR, androgen receptor.

Table 3
Protein-coding genes regulate chemoresistance of bladder cancer in experimental studies.

Coding genes	Expression in tumor	Drug	Regulation mechanism	Ref.
β-arrestins	β-arrestin-1 upregulated; β-arrestin-2 downregulated	Gemcitabine	β-arrestin-2 reduces expression of CSCs markers, β-arrestin-1 has opposite effects	[96]
IGF-1 FGFR3c	NA	Cisplatin	CAFs increase IGF-1/ERβ/Bcl-2 to promote cisplatin resistance	[107]
	Upregulated	Cisplatin	P4 binds FGFR3c to abrogate the suppression effects of FGF9 on cell apoptosis to increase cisplatin sensitivity	[111]
TACC3	Upregulated	Cisplatin	TACC3 activates E2F1 transcription to promote G1/S transition and enhance the sensibility to cisplatin	[115]
ELK1	Upregulated (phosphorylated form)	Cisplatin	PKC/Raf-1/ERK targets ELK1 to contribute to cisplatin sensitivity	[118]
CHK1	NA	Gemcitabine	AZD7762 inhibits CHK1 to suppress the repair of gemcitabine-induced double strand breaks	[119]
Maspin	Downregulated	Cisplatin	Maspin regulates PI3K/Akt, mTOR, and caspase pathways, to enhance apoptosis.	[121]
hnRNPK	Upregulated	Cisplatin	hnRNPK promotes anti-apoptosis and chemoresistance via regulating transcription of cyclin D1, GOS2, XAF1 and ERCC4	[122]

CSCs, cancer stem cells; CAFs, cancer-associated fibroblasts; IGF-1, insulin-like growth factor-1; IGF-1R, insulin-like growth factor-1 receptor; ERβ, estrogen receptor beta; FGF9, fibroblast growth factor 9; FGFR3c, fibroblast growth factor 3c; TACC3, transforming acidic coiled-coil protein 3; CHK1, checkpoint kinase 1 Maspin, Mammary serine protease inhibitor; hnRNPK, heterogeneous nuclear ribonucleoprotein K.

Targeting DNA damage repair and anti-apoptosis genes

It is widely known that cisplatin induces cytotoxic effects by driving DNA damage. Poly(adenosine diphosphate [ADP]) ribose polymerases (PARPs) participate in the DNA damage repair (DDR) process. Thus, PARP inhibitors lead to DNA double-strand breaks that are normally repaired by the homologous recombination repair mechanism in the late S or G2 phase of the cell cycle, which has shown attractive results in other types of tumours, such as ovarian, breast and prostate cancers [61]. Although genes known

to be associated with PARP inhibitor sensitivity (CHEK1/2, RAD51, BRCA1/2, ATM, ATR, MDC1 and FANCF) identified in 34% of bladder cancers and approximately 60% of urothelial carcinoma patients preserve homologous recombination deficiency, PARP inhibitor monotherapy shows no significant activity in advanced urothelial carcinoma regardless of homologous recombination deficiency status [62,63]. Interestingly, defects in ATM, RB1 and FANCC, which are DDR genes, have been identified as biomarkers of neoadjuvant cisplatin sensitivity in bladder cancer [64]. However, few studies have assessed whether the addition of PARP inhi-

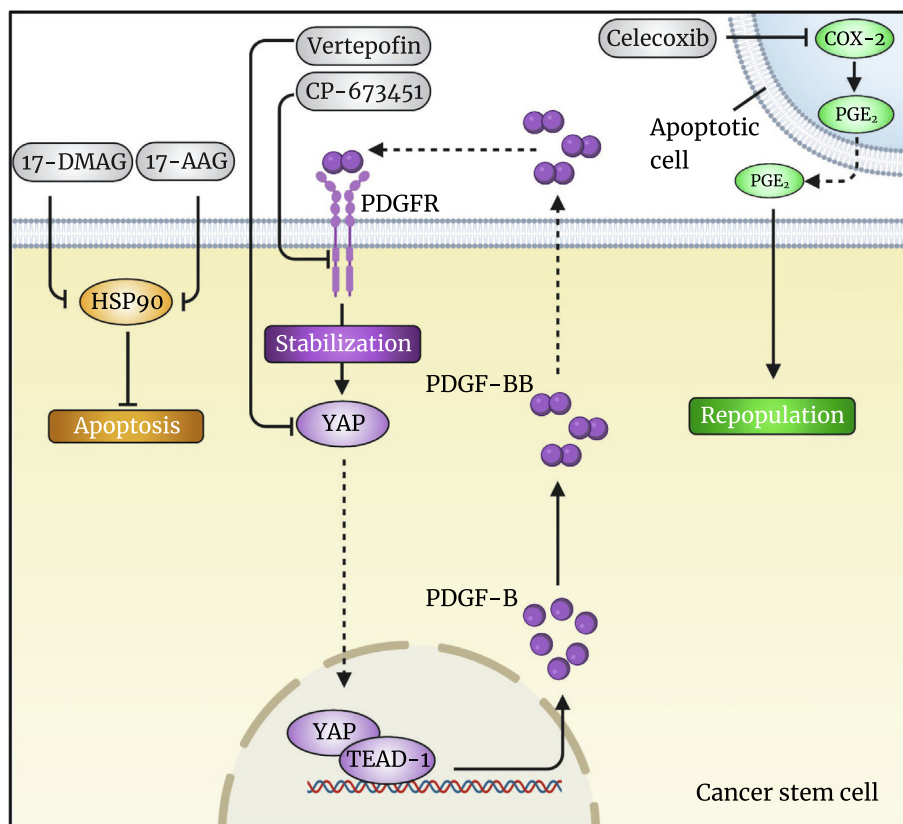


Fig. 1. Cancer stem cells contribute to chemosensitization of bladder cancer in preclinical studies. HSP90, heat shock protein 90; PDGFR, platelet-derived growth factor receptor; PDGF-B, platelet-derived growth factor-B; PDGF-BB, platelet-derived growth factor-B dimer; YAP, yes-associated protein; TEAD, TEA domain transcription factor.

bitors to cisplatin-based chemotherapy achieve a significant improvement in bladder cancer treatment, which may be a future direction to enhance chemotherapy efficacy. ERCC2, another gene that contributes to nucleotide excision repair of the DDR process, repairs the intrastrand crosslinks created by platinum chemotherapies to develop resistance [65]. A novel functional assay has identified mutations in the ERCC2 helix domain that damage its own function of nucleotide excision repair [66], and an ERCC2 mutation has been introduced into a cell line, sensitizing an orthotopic xenograft model of bladder cancer to cisplatin [66].

Given that both the DDR and antiapoptosis give rise chemoresistance, identification of the upstream regulatory mechanism of these proteins is urgent and important. WD repeat domain 5 (WDR5), a key H3K4 methyltransferase, plays an oncogenic role in cisplatin chemoresistance in bladder cancer by regulating DNA damage repair and antiapoptotic genes [67]. Furthermore, the WDR5 inhibitor, OICR-9429, enhances apoptosis and chemosensitivity to cisplatin in bladder cancer by blocking the WDR5-MLL complex mediating H3K4me3 in target genes, especially BIRC5, XRCC2 and AURKA. In addition, targeting WDR5 by OICR-9429 also suppresses proliferation and metastasis [68]. A similar result has also been found in prostate cancer [69]. These studies suggest that OICR-9429 is a multipotency anticancer therapy that enhances the antitumour effect of cisplatin in bladder cancer (Fig. 2).

Targeting oncogenes and tumour suppressor genes

Recently, it has been found that regulating some well-known molecules and pathways that have been previously reported to participate in cancer initiation and progression may potentiate the anticancer activity of cytotoxic agents. The mutation of RAS abrogates its GTPase activity, which causes GTP-bound RAS to con-

stitutively trigger a signalling cascade and lead to tumour progression [70]. HRAS is one of the isoforms of RAS proteins, which is mutated in nearly 15% of bladder cancer cases [71]. Chen *et al* elucidated that pterostilbene, a polyphenol phytoalexin, sensitizes cisplatin-resistant bladder cancer cells to oncogenic HRAS via autophagy and cell senescence *in vitro* and *in vivo* [72]. In addition, as a p53 homologue, the transactivation domain of p73 (TAp73) also contributes to bladder cancer development as a tumour suppressor [73]. Furthermore, pretreatment with 1,25D₃ induces p73 to potentiate the efficacy of GC *in vitro* and *in vivo* in bladder cancer [74].

Targeting receptors

Epidermal growth factor receptor (EGFR) is a broadly investigated target in cancer treatment and is also associated with muscle invasion and poor tumour differentiation in bladder cancer [75]. Tyrosine kinase inhibitors and monoclonal antibodies have long been introduced to inhibit EGFR to generate antitumour effects. Wang *et al* demonstrated that the EGFR/STAT3 pathway triggers cisplatin-induced CCAAT/enhancer-binding protein delta (CEBPD) expression. Elevated CEBPD activates multidrug resistance transporters, including ATP binding cassette subfamily B member 1 (ABCB1) and ATP binding cassette subfamily C member (ABCC2), leading to the cross-resistance of cisplatin and paclitaxel [76]. Cross-resistance is suppressed by gefitinib (an EGFR inhibitor) or S3I-201 (a STAT3 inhibitor) *in vitro* and *in vivo* (Fig. 1), suggesting the potential of administering EGFR inhibitors to bladder cancer patients using cisplatin and paclitaxel.

Increased survival signals of androgen receptor (AR) and NF-κB have been identified in MIBC via tumour tissue microarrays [77]. ASC-J9, an AR degradation enhancer without damaging libido, fer-

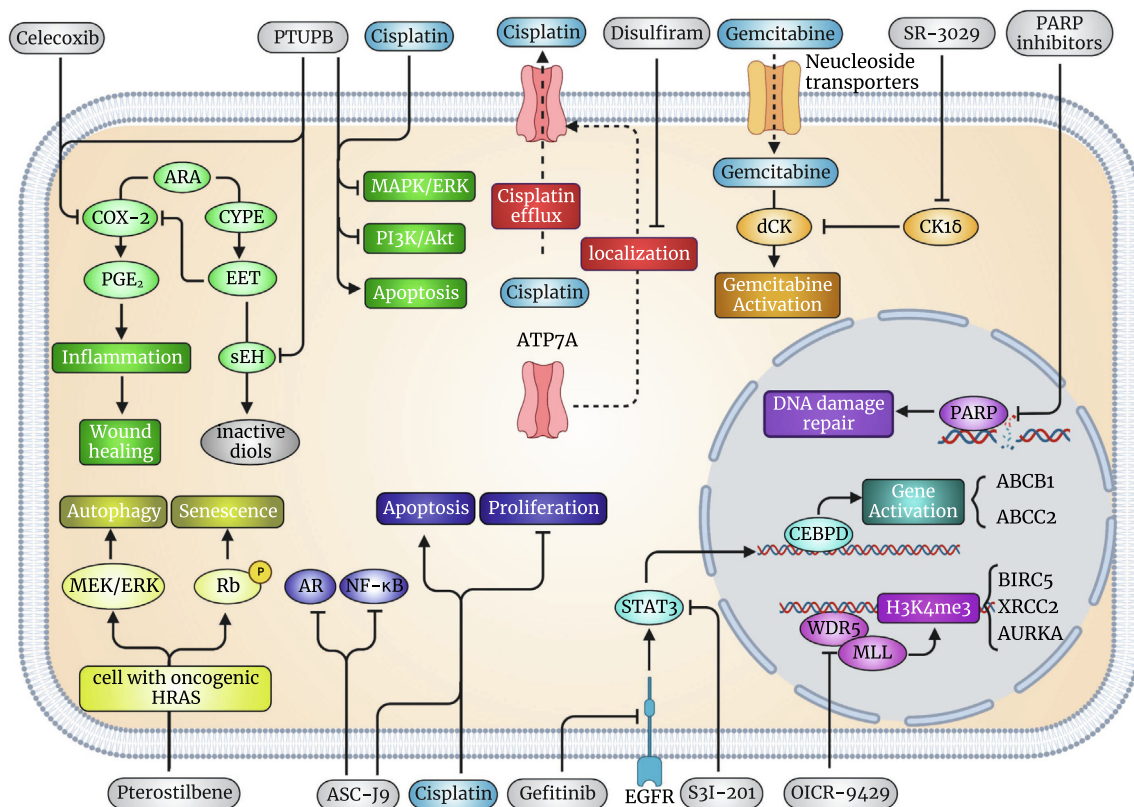


Fig. 2. Promising targets and pathways that improve the chemotherapy efficacy of bladder cancer in preclinical studies. ARA, arachidonic acid; COX-2, cyclooxygenase-2; CYP2, cytochrome P450 epoxygenase; PGE₂, prostaglandin E₂; EET, epoxyeicosatrienoic acid; sEH, soluble epoxide hydrolase; CK1 δ , casein kinase 1 delta; dCK, deoxycytidine kinase; PARP, poly(adenosine diphosphate [ADP]) ribose polymerases; WDR5, WD repeat domain 5; H3K4me3, trimethylation of lysine 4 on histone H3 protein subunit; EGFR, epithelial growth factor receptor; CEBP Δ , CCAAT/enhancer-binding protein delta; AR, androgen receptor.

tility and sexual behaviour, was first applied as a topical cream for the treatment of acne vulgaris. Combining cisplatin with ASC-J9 suppresses bladder cancer progression better than cisplatin alone *in vivo*. Mechanistically, the combined therapy not only promotes the degradation of AR but also diminishes the activation of NF- κ B in MIBC cells. The inhibition of AR and NF- κ B signals increases the expression of pro-apoptosis genes, including BAX and p21, while it decreases the expression of the Bcl-2 pro-survival gene. Nevertheless, establishment of the safety profile of ASC-J9 as a systemic agent is warranted [77,78].

Regulating tumour immunology

Immune escape is one of the major processes that occur during bladder cancer tumorigenesis and progression. Immunotherapy has achieved favourable results in the management of bladder cancer. PD-1/PD-L1 is one of the most well-known molecular pairs that contribute to bladder cancer progression [79]. Generally, PD-L1 is expressed on the surface of bladder cancer cells to inhibit the activation of T cells when it binds to PD-1, its receptor expressed on T cells. Hence, ICIs are introduced based on the antitumour activity of inhibiting PD-1/PD-L1. Despite the antitumour effect of anti-PD-1/PD-L1 when it is administered as monotherapy, it has a potential future in overcoming chemoresistance of bladder cancer based on the results of clinical trials, which has been reviewed in the above section. Other agents may also target PD-1/PD-L1 to enhance the efficacy of chemotherapy. The aforementioned small molecule compound, OICR-9429, a WDR5 inhibitor, also regulates the expression of PD-L1 and potentiates chemosensitivity in bladder cancer [68]. Mechanistically, OICR-9429 inhibits WDR5-MLL complex-mediated H3K4me3, resulting in decreased

transcription of PD-L1. Hence, OICR-9429 suppresses immune evasion by blocking PD-L1, ultimately enhancing cisplatin chemosensitivity [68].

Xenotransplantation was designed to compensate for the shortage of donors in organ transplantation, but it faces challenges due to the xenogeneic immune reaction. Conversely, the xenogeneic immune reaction is a potential way to overcome immune tolerance induced by cancer cells. Hence, xenovaccination has become another effective immunotherapy strategy. Huang *et al* reported a novel intravesical application of xenogeneic urothelial cells isolated from porcine bladders combined with GC chemotherapy in bladder tumour mouse models [80]. The combined therapy prolongs survival time in the orthotopic MBT-2 graft model and suppresses tumour progression in the BBN-induced tumour model. Mechanistically, the application of intravesical xenogeneic urothelial cells stimulates lymphocytes to release more IFN- γ and significantly enhances the capacity of CD8⁺ cytotoxic T cells. The results of this study support the possibility that xenogeneic urothelial cells may be regarded as a novel immunotherapeutic agent alone or used in combination with chemotherapy for the management of bladder cancer.

Nanotechnology

Novel drug delivery systems, including nanoparticles and liposomes, have been designed to optimize pharmacokinetics in cancer treatment, thus showing better antineoplastic effects by directly targeting cancer cells and modulating the tumour microenvironment (TME). A recent study has reported a considerable effect of combining gemcitabine nanoparticles and cisplatin nanoparticles as an antitumour therapy by targeting cancer-associated fibrob-

lasts (CAFs) [81]. The combined nanoparticles of GC show a stronger inhibition of tumour growth and less toxicity than single GC. Mechanistically, the effect of combined nanoparticles increases the uptake of drugs into cancer cells and depletes CAFs with alterations in collagen deposition. Interestingly, although cisplatin nanoparticles injure CAFs and inhibit tumour progression initially, long-term application of the nanoparticles increases the secretion of Wingless-type MMTV integration site family member 16 (Wnt16) in a paracrine fashion, leading to chemoresistance and stroma reconstruction [82]. These results suggest that inhibition of Wnt16 might have a synergistic effect with cisplatin nanoparticle therapy of bladder cancer.

Nanoparticles have also been reported to be combined with gas therapy to sensitize patients to chemotherapy. Recently, a photoactivated hydrogen nanogenerator has been introduced. The novel agent is a self-assembled nanoparticle containing gemcitabine with the ability to produce hydrogen gas upon laser irradiation *in situ* of the bladder [83]. Hydrogen gas has been previously attributed to contribute to antioxidant, antiapoptotic and antitumour activities in combination with nanomedicine [84–86]. The intravesical instillation of the hydrogen nanogenerator effectively penetrates tumour cells and then releases hydrogen gas to significantly enhance chemotherapy *in vitro* and *in vivo*, mainly by attenuating P-gp capacity [83].

Regarding liposomes, Zhai *et al* developed a targeted liposome to deliver β -elemene into urokinase plasminogen activator receptor (uPAR)-overexpressing bladder cancer cells [87]. An amino-terminal fragment (ATF) peptide, which competes with urokinase plasminogen to bind with uPAR, is assembled to construct the ATF₂₄ peptide-functionalized β -elemene-nanostructured lipid carrier. The combination of uPAR-targeted β -elemene liposomes and cisplatin exerts a synergistic effect on apoptosis and cell cycle arrest, resulting in inhibition of tumour growth [87].

Although nanomedicines have been investigated and applied for years, the following challenges still exist: immune clearance by the liver and spleen; the efficacy of permeation and penetration to the stroma; and endocytosis and diffusion in target cells [88]. Second-generation nanomedicines with active-targeting vehicles or smart vectors with stimuli-responsive properties are being investigated to enhance their efficacy [88]. Furthermore, nanotechnology has shown advantages for intravesical chemotherapy [89]. Hydrogels have been designed to prolong the residence time and sustain the release of drugs, resulting in maintenance of the local drug concentration [90]. For example, a PEG-containing thermogel has been developed for combination doxorubicin-based chemotherapy and photodynamics to treat bladder cancer after transurethral resection [91]. Additionally, engineered nanomedicines have been introduced to improve tumour penetration [92]. In bladder cancer, Guo *et al* developed a smart disulfide-crosslinked polypeptide nanogel to promote the mucoadhesion and penetrability of 10-hydroxycamptothecin for intravesical chemotherapy [93,94]. Evolution in nanomedicine is rapid, and sequentially stimuli-responsive anticancer nanomedicines are the cutting edge [95]. The integration of sequentially stimuli-responsive nanomedicines and chemotherapy in both intravesical and systemic treatment of bladder cancer should be further investigated.

Potential targets of chemosensitization in experimental studies

Apart from the aforementioned molecules and pathways that have been targeted to potentially overcome chemoresistance of bladder cancer, other coding genes (Table 3 and Fig. 3) and noncoding RNAs (Table 4) also contribute to sensitization of chemoresistance by regulating cancer cell metabolism, CSC functions and other phenotypes. Although these findings are still preliminary,

they provide ideas for the discovery of novel drugs that target these potential pathways.

Cancer stem cell-related pathways

We reviewed drugs that regulate CSCs and contribute to sensitization to cisplatin resistance in bladder cancer. The CSC-associated pathway has also been suggested to regulate the response to gemcitabine in bladder cancer. β -arrestins that contain β -arrestin-1 and β -arrestin-2 attenuate G-protein-coupled receptor signalling. β -arrestins regulate the stem cell-like phenotype and the response to gemcitabine in bladder cancer [96]. In bladder cancer tissues, β -arrestin-1 is upregulated, while β -arrestin-2 is downregulated. Overexpression of β -arrestin-2 reduces the expression of CSC markers and potentiates sensitivity to gemcitabine *in vitro* and *in vivo*, while β -arrestin-1 has the opposing effect [96]. Thus, β -arrestins act as potential prognostic indicators and targets for the identification and modification of the gemcitabine response.

Noncoding RNAs are also implicated in enhancing chemosensitivity by regulating CSCs. Accumulating evidence has suggested a critical role of miR-34a in bladder cancer chemosensitization. MiR-34a was first found to be epigenetically increased via promoter hypermethylation in MIBC cells following cisplatin treatment [97]. The increased miR-34a targets CD44 and reduces its expression, which in turn sensitizes MIBC cells to cisplatin [97]. Additionally, a decreased expression level of miR-34a in GC-resistant cell lines has been reported [98]. MiR-34a abrogates CSC characteristics by inhibiting GOLPH3, resulting in resensitization of GC cells *in vitro* and *in vivo* [98]. In addition, miR-34a also regulates sensitivity to cisplatin and epirubicin in bladder cancer via other mechanisms, which will be discussed in later sections. Regarding lncRNAs, Chen *et al* identified a lncRNA, termed lnc-LBCS, that is downregulated in bladder CSCs and tumour tissues, and they reported that lnc-LBCS is associated with chemotherapy response and prognosis [99]. lnc-LBCS was discovered to inhibit chemoresistance by directly binding to heterogeneous nuclear ribonucleoprotein K (hnRNPK) and enhancer of zeste homologue 2 (EZH2), thereby repressing SRY-Box 2 (SOX2) transcription by mediating histone H3 lysine 27 trimethylation [99]. The effect of chemosensitization of lnc-LBCS has been further confirmed *in vivo*, indicating that the lnc-LBCS/hnRNPK/EZH2/SOX2 axis may provide a potential target for overcoming chemoresistance in bladder cancer [99]. Furthermore, EZH2 is also silenced by miR-101-3p to increase the sensitivity to cisplatin in bladder cancer [100].

Tumour microenvironment

The acquisition and maintenance of the hallmarks of cancer have been demonstrated to be dependent, to various degrees, on the contributions from nonmalignant cells in tumours, including stromal cells (e.g., cancer-associated fibroblasts and endothelial cells) and immune cells [101]. These noncancerous cells and their extracellular milieu form the tumour microenvironment (TME) and interact with cancer cells to play key roles in bladder cancer progression, metastasis and therapeutic responses [102–105]. The application of ICIs is the landmark of targeting TME-cancer cell interactions to inhibit cancer progression. Intriguingly, the diversity and complexity of the cell subpopulations in tumours have been unmasked due to the development of single-cell sequencing technology. Furthermore, components of the TME mediate the response to selection pressure of cytotoxic agents [106], but the mechanisms remain unknown. As a key component of the TME, cancer-associated fibroblasts (CAFs) promote cisplatin resistance in bladder cancer by triggering the insulin-like growth factor-1/

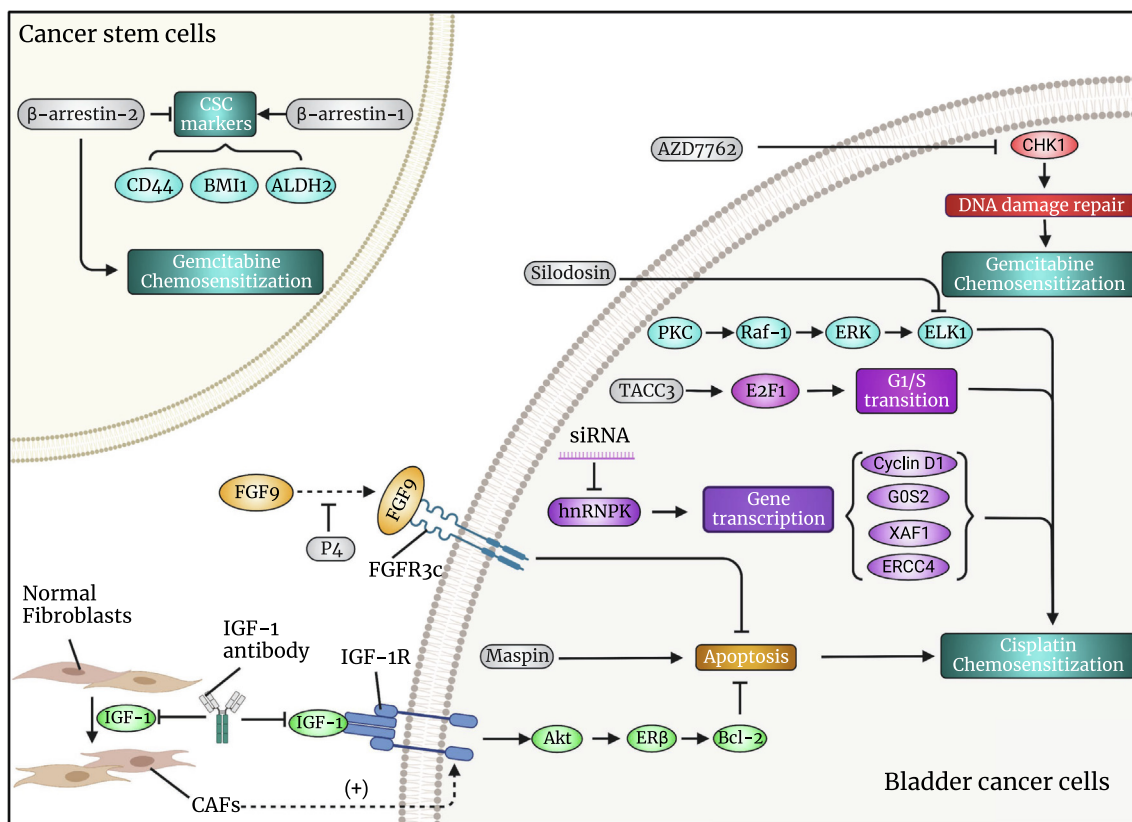


Fig. 3. Targets and pathways that potentially sensitize bladder cancer patients to chemotherapy in experimental studies. CSC, cancer stem cell; CAFs, cancer-associated fibroblasts; IGF-1, insulin-like growth factor-1; IGF-1R, insulin-like growth factor-1 receptor; ER β , oestrogen receptor beta; Maspin, mammary serine protease inhibitor; FGF9, fibroblast growth factor 9; FGFR3c, fibroblast growth factor 3c; hnRNPK, heterogeneous nuclear ribonucleoprotein K; TACC3, transforming acidic coiled-coil protein 3; CHK1, checkpoint kinase 1.

oestrogen receptor β (IGF-1/ER β) pathway, which elevates the expression of Bcl-2, an antiapoptotic gene [107]. Shan *et al* found that exosomal miR-148b-3p derived from CAFs is downregulated in bladder cancer, which inhibits the Wnt/ β -catenin pathway and increases PTEN expression to enhance chemosensitivity *in vitro* and *in vivo* [108]. These results suggest that targeting CAFs may be an effective therapeutic strategy to ameliorate chemoresistance in bladder cancer. Other components of the TME also contribute to the plasticity of chemotherapy-treated cancer cells. The mechanisms by which the TME regulates chemosensitivity and targeting of the TME to potentiate the efficacy of chemotherapy warrant further investigation.

Fibroblast growth factor receptor

The fibroblast growth factor (FGF) family has been reported to play essential roles in oncogenesis and tumour progression [109]. Erdafitinib, a pan-FGFR inhibitor, was the first approved targeted agent by the FDA for the treatment of metastatic urothelial carcinoma [110]. FGFR3c, one of the subtypes of FGFR, is overexpressed in bladder cancer [111]. Wang *et al* isolated a binding peptide of FGF9 from a phage display random heptapeptide library, termed P4, and they reported that P4 increases sensitivity to cisplatin by antagonizing the FGF9/FGFR/PI3K/Akt pathway [112]. Interestingly, transforming acidic coiled-coil protein 3 (TACC3), which participates in mitosis by regulating microtubule stability, has been reported to fuse to FGFR3 by chromosomal arrangement and continuously activate kinase [113]. TACC3 expression is elevated in bladder cancer, and a genome-wide association study (GWAS) has identified TACC3 as a cancer susceptibility gene of bladder can-

cer [114,115]. Lin *et al* demonstrated that TACC3 transcriptionally activates E2F1, thereby promoting the G1/S transition to enhance the sensitivity to cisplatin [115].

Apoptosis and cell cycle-related coding genes

The combined application of introduced drugs and compounds has been investigated to show the potential efficacy of sensitizing bladder cells against chemoresistance by regulating apoptosis and the cell cycle. Triptolide, an extract from Chinese herbal medicine, has been reported to have antineoplastic effects [116]. Triptolide combined with cisplatin induces a synergistic cytotoxic effect via cell cycle arrest and the upregulation of caspase-3/8/9, PARP and cytochrome C in a cisplatin-resistant T24 cell line [117].

Silodosin is a selective α 1A-adrenergic blocker for the treatment of benign prostatic hyperplasia, and it has been found to increase drug sensitivity to cisplatin but not to gemcitabine in bladder cancer [118]. Mechanistically, silodosin silences ELK1, which has been identified to be elevated in bladder cancer cell lines and tumour tissues, and diminishes NF- κ B *in vitro* [118]. A checkpoint kinase 1 (CHK1) inhibitor, termed AZD7762, also sensitizes bladder cancer cells to gemcitabine by increasing the fraction of sub-G1 cells, the level of cleaved PARP and the activity of caspase 3/7 to induce apoptosis [119].

Some regulators of apoptosis and cell cycle-related pathways also show the potential to improve the efficacy of chemotherapy. Mammary serine protease inhibitor (maspin) expression is significantly reduced in invasive bladder cancer compared to superficial bladder cancer, and it is correlated with the prognosis of patients who have received cisplatin-based neoadjuvant chemotherapy

Table 4
Noncoding RNAs sensitize chemoresistance of bladder cancer in experimental studies.

Non-coding RNAs	Expression in tumor	Target Gene	Drug	Regulation mechanism	Ref.
microRNA					
miR-34a	Downregulated (in nonresponders)	CDK6, SIRT1	Cisplatin	As a downstream effector of p53 to inhibit expression of CDK6 and SIRT1	[123]
miR-34a	Upregulated (After cisplatin treatment)	CD44	Cisplatin	Cisplatin-based chemotherapy induces demethylation of miR-34a and increases its expression which targets CD44	[97]
miR-34a	Downregulated (in GC resistant cell lines)	GOLPH3	GC	miR-34a/GOLPH3 abrogates chemoresistance via reduced cancer stemness	[98]
miR-34a	Downregulated	TCF1, LEF1	Epirubicin	As an inhibitor of TCF1/LEF1 axis	[124]
miR-34b-3p	NA	CCND2, P2RY1	Paclitaxel, Adriamycin, Epirubicin, Cisplatin, Pirarubicin	miR-34b-3p attenuates chemoresistance suppressing CCND2 and P2RY1	[125]
miR-101-3p	Downregulated (in cisplatin resistant cell lines)	EZH2	Cisplatin	miR-101-3p advances sensitivity to cisplatin through targeted silencing EZH2	[100]
miR-129-5p	Downregulated (in cisplatin resistant tissues)	Wnt5a	Gemcitabine	restoration of miR-129-5p increases cell sensitivity to gemcitabine by targeting Wnt5a	[128]
miR-143	Downregulated	IGF-1R	Gemcitabine	miR-143 enhances gemcitabine sensitivity via IGF-1R suppression	[129]
miR-148b-3p	Downregulated (in CAF-derived exosomes)	PTEN	Doxrubicin, Paclitaxel	miR-148b-3p inhibits the Wnt/ β -catenin pathway and promoting PTEN expression to abrogate drug resistance	[108]
miR-203	Downregulated (in progression group)	Bcl-w, Survivin	Cisplatin	miR-203 overexpression enhances cisplatin sensitization by promoting apoptosis via targeting Bcl-w and Survivin	[126]
miR-214	Downregulated	Netrin-1	Cisplatin	miR-214 decreases chemoresistance by suppressing Netrin-1	[127]
miR-218	NA	Glut1	Cisplatin	miR-218 reduces the rate of glucose uptake and total level of GSH and enhances the chemosensitivity via targeting GLUT1	[137]
long non-coding RNA					
lnc-LBCS	Downregulated (in CSCs)	SOX2	GC	As a scaffold to form the complex of lnc-LBCS/hnRNPK/EZH2 to repress SOX2 transcription via H3K27me3 in CSCs	[99]
GAS5	Downregulated	Bcl-2	Doxorubicin	GAS5 increases doxorubicin-induced apoptosis through Bcl-2 suppression	[130]
circular RNA					
Cdr1as	NA	APAF1	Cisplatin	As a miRNA sponge to regulate miR-1270/APAF1 axis	[131]
circFNTA	Upregulated	FNTA	Cisplatin	circFNTA regulates miR-370-3p/FNTA/KRAS axis to enhance chemoresistance	[134]
circLIFR	Downregulated	p73	Cisplatin	Interacting with MSH2 to increase cisplatin sensitivity through MutS α /ATM-p73 axis	[133]

NA, not available; GC, gemcitabine plus cisplatin; CAF, cancer-associated fibroblast; CSCs, cancer stem cells; H3K27me3, trimethylation of lysine 27 on histone H3 protein subunit.

[120,121]. Chen *et al* found that maspin potentiates the sensitivity of T24 and 5637 cells to cisplatin by regulating the PI3K/Akt, mTOR and caspase pathways, resulting in upregulation of cisplatin-induced apoptosis [121]. Heterogeneous nuclear ribonucleoprotein (hnRNPK) is a key regulator of DNA transcription. Silencing hnRNPK by siRNA represses cisplatin resistance by regulating the transcription of cyclin D1, GOS2, XAF1 and ERCC4 *in vitro*, suggesting that hnRNPK is a promising target to sensitize chemotherapy [122].

Non-coding RNAs regulating apoptosis and cell cycle

Apart from regulating CSC function, miR-34a inhibits CDK6 to regulate the p53-Rb axis to enhance chemosensitivity to cisplatin [123]. Furthermore, Liu *et al* also discovered that miR-34a directly targets TCF and LEF1, which are involved in the Wnt/ β -catenin pathway, to reduce chemoresistance both *in vivo* and *in vitro* [124]. Other miRNAs, including miR-34b-3p [125], miR-203 [126], miR-214 [127], miR-129-5p [128] and miR-143 [129], have been reported to attenuate chemoresistance by targeting different mRNAs to regulate apoptosis and the cell cycle in bladder cancer cells. Details of these miRNAs are summarized in Table 4.

For lncRNAs and circRNAs, the regulation of apoptosis and the cell cycle are also common mechanisms by which they regulate chemoresistance in bladder cancer. lncRNA GAS5 decreases the expression of Bcl-2 to promote apoptosis induced by doxorubicin in a doxorubicin-resistant cell line [130]. CircRNA Cdr1as sponges miR-1270 to abolish its inhibitory effect on apoptosis protease-activating factor 1 (APAF1), which is a key factor that regulates apoptosis, thereby promoting sensitization to cisplatin *in vitro* and *in vivo* [131]. MutS homologue 2 (MSH2) is a mediator of cisplatin sensitivity [132]. A downregulated circRNA in bladder cancer, termed CircLIFR, has been reported to interact with MSH2 to stabilize p73, a key trigger of apoptosis, thereby augmenting the sensitivity to cisplatin in bladder cancer [133]. We reviewed that AR and RAS are targeted to increase sensitivity to cisplatin. Surprisingly, Chen *et al* found that inhibition of circFNTA regulates the miR-370-3p/FNTA/KRAS axis to sensitize cisplatin chemotherapy *in vitro* and *in vivo* [134].

Glucose metabolism

Cancer cells prefer using glucose to produce lactate (i.e., glycolysis) even under oxygen-rich conditions, which is widely known as the “Warburg effect” [135]. Glycolysis-associated genes, such as

glucose transporter type 1 (GLUT1), enhance glycolytic activity and contribute to cancer progression; GLUT1 is overexpressed in bladder cancer [136]. MiR-218 increases the sensitivity of bladder cancer by targeting GLUT1 *in vitro* [137]. Casein kinase 2 (CK2) also augments the Warburg effect and promotes the proliferation, migration and invasion of cancer cells [138], of which CK2 α is the essential catalytic subunit [139]. AlkB homologue 5 RNA demethylase (ALKBH5) has been reported to sensitize cisplatin resistance through a CK2 α -mediated glycolysis pathway. These findings suggest that epigenetic regulation of glycolysis pathways may alter the “Warburg effect” and potentially attenuate chemoresistance.

Application of patient-derived tumour xenografts (PDXs) and organoids in chemosensitization and targeted therapy

Due to the heterogeneity of tumours, none of the agents or regimens exert comparable effects in different patients. Identifying biomarkers and testing the sensitivity of chemotherapeutic agents in models derived from tumour tissues of specific patients are promising approaches. The present platforms have originated from primary tumour tissues, including patient-derived xenografts (PDXs) and organoids, and these models have the potential to design sensitive drugs for bladder cancer patients. First, genomic profiling of two PDXs derived from two bladder cancer patients with different responses to cisplatin indicated distinct profiles [140]. In the less sensitive PDX, nonsense mutations in cisplatin resistance-associated genes and the overexpression of other cisplatin resistance-associated genes were identified, suggesting that specific gene alterations from the PDX may be predictive of cisplatin sensitivity [140]. PDXs simulate tumour characteristics *in vivo* and preserve the TME, but PDXs are difficult to generate and utilize for high-throughput screening of drugs. An *ex vivo* tissue culture model generated from bladder cancer tissue slices from a transurethral resection of a bladder from a NMIBC patient has been reported to be more applicable; the tissue slices can be cultured in the presence of gemcitabine to identify the level of cleaved caspase-3 and the number of cytokeratin-18-positive tumour cells, which can be exploited to monitor the gemcitabine response [141].

Organoids are three-dimensional *in vitro* culture systems derived from self-organizing stem cells that recapitulate the *in vivo* architecture, functionality and genetic signature of primary tissues. Organoids of bladder cancer have been established and developed to predict drug responses to targeted therapy and chemotherapy [142–144]. Kong *et al* conducted a machine-learning framework to identify robust biomarkers from pharmacogenomic data derived from organoids [144]. The identified biomarkers have been reported to predict cisplatin responses of 77 bladder cancer patients [144]. Although the structure of organoids is simpler than that of PDXs, it is difficult to simulate the circulation and metabolism of drugs *in vivo*. Additional incorporation of immune components in organoids will increase the sophistication while preserving the advantages of easy culturing and expansion, allowing more advanced estimations of drug responses.

Future perspectives for improving chemotherapy in bladder cancer

Platinum-based chemotherapy has long been the first-line treatment of MIBC with definite effects of downstaging and prolonging OS and PFS. Unfortunately, limited patients receive considerable advantages, while others delay operations and suffer from chemotherapy-associated adverse effects. Thus, developing novel strategies to improve the efficacy of chemotherapy is important. In this review, we summarized novel regimens, drugs and targets

that exert the potential of chemosensitization based on the results of clinical trials and preclinical and experimental studies. In clinical trials, monotherapy and/or a combination of ICIs, ADCs and VEGF inhibitors has shed light on the potential of elevating the response rate as well as increasing OS and PFS, providing high-level evidence of potentiating chemosensitivity. Specifically, PD-1/PD-L1/CTLA-4 inhibitors are presently available for the clinical treatment of bladder cancer and other cancer types, indicating their controllable adverse effects and increased application experience compared to novel generated compounds, such as targeted drugs. Thus, the good performance of these inhibitors in ameliorating chemoresistance is the beginning for establishing a novel clinically applicable regimen. Clinicians and researchers should pay more attention to the application of these ICIs along with chemotherapy drugs in ongoing phase 2 and 3 clinical trials. For preclinical and experimental studies, massive prospects have been exhibited in targeting critical mechanisms and pathways of chemoresistance, including CSCs, DNA damage repair, antiapoptosis, drug metabolism and the TME. In addition to abrogating chemoresistance by applying small molecule inhibitors, targeting noncoding RNAs is also a new approach that is worth further development and verification. Pharmacokinetics, as an inherent characteristic of an introduced drug, is not easy to optimize by changing the structure of the compound without affecting the therapeutic effects and safety. Importantly, packing drugs in nanoparticle carriers enhances their accumulation in tumours, resulting in better cytotoxic effects in tumours and less damage to unrelative tissues. Present studies mainly focus on potentiating antitumour effects through nanotechnology, but we suggest that the ability to attenuate chemotherapy-associated adverse effects is also essential. Less systemic damage from cytotoxic drugs may help patients tolerate postchemotherapy operations and allow an easier to recovery. More data comparing the adverse effects of nanoparticle-packed chemotherapeutic drugs to unpacked drugs are required.

Further illustration of the molecular mechanism of chemoresistance will provide more targets and biomarkers. To attenuate chemoresistance, efforts should be devoted to the following aspects. Considering that bladder cancer is a heterogeneous disease, multi-omics sequencing and analysis may contribute to more robust biomarkers for chemosensitivity prediction. Gene expression models and/or the molecular subtypes established from the data of multi-omics sequencing may screen specific groups of patients who may achieve a favourable response to cytotoxic agents. Synergic targeting of the dysregulated molecular pathways that are explicitly identified in patients resistant to chemotherapy has the potential to reverse the resistance. In addition to cancer cells, other components of the stroma and/or TME, such as immune cells and CAFs, are also worth targeting to regulate chemoresistance-associated tumour-TME interactions. It is worth investigating whether multitarget intervention sensitizes patients to chemotherapy by using PDXs and/or organoids. Nanotechnology and ADCs have the potential for precise delivery of drugs. All the above strategies aim to overcome the barriers of limited curative rate and undesired adverse effects, resulting in improved response rate and increased OS and PFS (Fig. 4).

In addition to chemotherapy, the efficacy of all introduced agents should be improved. It is important to realize that all kinds of drugs are only effective for a certain portion of patients. To date, the response of patients can only be determined after the application of therapeutic drugs. Thus, one of the remaining challenges for the treatment of bladder cancer is to identify the specific population sensitive to a certain agent prior to its administration using blood, urine and tumour samples. Considering the characteristics of the tumour change under the pressure of therapeutic agents, the establishment of validation models, including PDXs and organoids, is important for the development of drug screening and val-

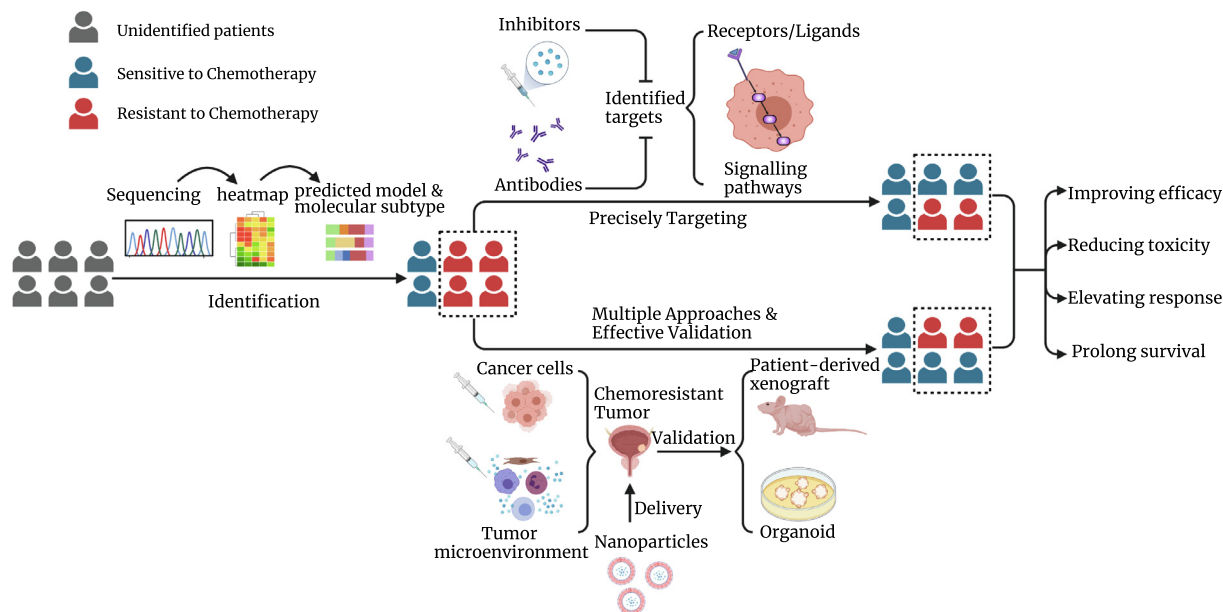


Fig. 4. Prospect of precision chemotherapy and targeted therapy for bladder cancer.

ication. Clinical trials are warranted to evaluate whether the response rate and/or survival are improved in the subpopulations of identified sensitive patients via selection by models compared to whole cohorts. As a heterogeneous disease, discovering critical targets for bladder cancer management is an indisputable approach to solve the dilemma of insensitivity of existing agents. Hence, the strategy of identification followed by precision targeting and effective validation by models is suitable and sustainable for most drugs at present as well as for the future.

Compliance with Ethics Requirement

Not Applicable.

CRedit authorship contribution statement

Sen Liu: Conceptualization, Writing – original draft, Writing – review & editing. **Xu Chen:** Conceptualization, Funding acquisition, Writing – review & editing. **Tianxin Lin:** Supervision, Funding acquisition, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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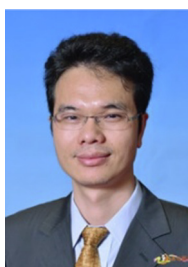
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